```
ost is in DialUnits
? b 410
       05jul10 08:11:26 User208760 Session D3198.1
            $0.58    0.154 DialUnits File1
     $0.58  Estimated cost File1
     $0.02  TELNET
     $0.60  Estimated cost this search
     $0.60  Estimated total session cost   0.154 DialUnits

File 410:The Chronolog 1991-2010/ Mar
       (c) 2010 Dialog. All rights reserved.

      Set  Items  Description
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? set hi ;set hi
HILIGHT set on as ''
HILIGHT set on as ''
? begin 5,73,155,399
       05jul10 08:11:35 User208760 Session D3198.2
            $0.00    0.115 DialUnits File410
     $0.00  Estimated cost File410
     $0.05  TELNET
     $0.05  Estimated cost this search
     $0.65  Estimated total session cost   0.269 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File   5:Biosis Previews(R) 1926-2010/Jul W1
         (c) 2010 The Thomson Corporation
  File  73:EMBASE 1974-2010/Jul 05
         (c) 2010 Elsevier B.V.
*File  73: The archive of Medline derived records was added to Embase.
  File 155:MEDLINE(R) 1950-2010/Jul 02
         (c) format only 2010 Dialog
*File 155: Medline has been reloaded. Please see HELP NEWS154
for information.
  File 399:CA SEARCH(R) 1967-2010/UD=15302
         (c) 2010 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
IPCR/8 classification codes now searchable as IC=. See HELP NEWSIPCR.

      Set  Items  Description
      ---  -----  -----------
? e au=deisseroth albert ?

Ref   Items  Index-term
E1        2  AU=DEISSEROTH AB
E2       61  AU=DEISSEROTH ALBERT
E3        0 *AU=DEISSEROTH ALBERT ?
E4      103  AU=DEISSEROTH ALBERT B
E5       38  AU=DEISSEROTH K
E6       61  AU=DEISSEROTH K.
E7      101  AU=DEISSEROTH KARL
E8        1  AU=DEISSEROTH WENDY
E9       13  AU=DEISSEROTH, A.
E10       6  AU=DEISSEROTH, A. B.
E11       1  AU=DEISSEROTH, AL
E12      66  AU=DEISSEROTH, ALBERT

          Enter P or PAGE for more
? s e1-e4
               2  AU=DEISSEROTH AB
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             61  AU=DEISSEROTH ALBERT
              0  AU=DEISSEROTH ALBERT ?
            103  AU=DEISSEROTH ALBERT B
      S1    166  E1-E4
? e au=zhang lixin ?

Ref   Items  Index-term
E1        3  AU=ZHANG LIXIAO
E2      269  AU=ZHANG LIXIN
E3        0 *AU=ZHANG LIXIN ?
E4        2  AU=ZHANG LIXIN LILLY
E5        1  AU=ZHANG LIXIN ZHU LIPING
E6       10  AU=ZHANG LIXING
E7        1  AU=ZHANG LIXING KAN GUANQING
E8        4  AU=ZHANG LIXIONG
E9        1  AU=ZHANG LIXUAN
E10      22  AU=ZHANG LIXUE
E11      12  AU=ZHANG LIXUN
E12       4  AU=ZHANG LIYA

          Enter P or PAGE for more
? s e2
      S2    269  AU='ZHANG LIXIN'
? s (S1 or s2) and (adenoviral or adenovirus)(20n)(vector?) and (Cd40L or cd154 or
cd40(w)ligand)
             166  S1
             269  S2
           42047  ADENOVIRAL
          136081  ADENOVIRUS
          694836  VECTOR?
           48856  (ADENOVIRAL OR ADENOVIRUS)(20N)VECTOR?
           10347  CD40L
            4611  CD154
           42860  CD40
          661511  LIGAND
           19956  CD40(W)LIGAND
      S3     12  (S1 OR S2) AND (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?)
                 AND (CD40L OR CD154 OR CD40(W)LIGAND)
? rd s3
      S4     10  RD S3  (unique items)
? t s4/3/all

 4/3/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
(c) 2010 The Thomson Corporation. All rts. reserv.

0021275602   BIOSIS NO.: 200900617039
Use of CD40L immunoconjugates to overcome the defective immune
  response to vaccines for infections and cancer in the aged
AUTHOR: Tang Yu Cheng; Thoman Marilyn; Linton Phyllis-Jean; Deisseroth
  Albert (Reprint)
AUTHOR ADDRESS: US FDA, Off Oncol Drug Prod, 10903 New Hampshire Ave,Bldg
  22,Room 6378, Silver Spring, MD 20993 USA**USA
AUTHOR E-MAIL ADDRESS: albert.deisseroth@yahoo.com
JOURNAL: Cancer Immunology Immunotherapy  58 (12): p1949-1957 DEC 2009 2009
ITEM IDENTIFIER: doi:10.1007/s00262-009-0718-3
ISSN: 0340-7004
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Abstract
LANGUAGE: English
```

4/3/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0020916731 BIOSIS NO.: 200900257065
TAA/ecdCD40L VPP Vaccination Induces Robust Adaptive Immune Response Even in Individuals with Post Transplantation Lymphopenia
AUTHOR: Tang Yucheng (Reprint); Park Yeon Hee; Maynard Jonathan; Li Pingchuan; Akbulut Hakan; Petersen Line; Deisseroth Albert B
AUTHOR ADDRESS: Sidney Kimmel Canc Ctr, San Diego, CA USA**USA
JOURNAL: Blood 112 (11): p141-142 NOV 16 2008 2008
CONFERENCE/MEETING: 50th Annual Meeting of the American-Society-of-Hematology San Francisco, CA, USA December 06 -09, 2008; 20081206
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

4/3/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0019615830 BIOSIS NO.: 200700275571
Subcutaneous injection of the Ad-sig-TAA/ecdCD40L adenoviral vector encoding a CD40ligand/tumor associated antigen secretory protein generates T cell dependent cellular immunity against tumor cell lines for up to one year.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin; Yuan Jing; Akbulut Hakan ; Maynard Jonathan; Linton Phyllis-Jean; Deisseroth Albert B
AUTHOR ADDRESS: Sidney Kimmel Canc Ctr, San Diego, CA USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 45 (Suppl. S): p282-283 MAR 2004 2004
CONFERENCE/MEETING: 95th Annual Meeting of the American-Association-for-Cancer-Research Orlando, FL, USA March 27 -31, 2004; 20040327
SPONSOR: Amer Assoc Canc Res
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

4/3/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18788546 BIOSIS NO.: 200600133941
Vector prime-protein boost vaccine induces immune response against "self-antigens" associated with epithelial neoplasms and tumor vascular endothelial cells.
AUTHOR: Tang Yucheng (Reprint); Maynard Jonathan; Akbulut Hakan; Linton Phyllis-Jean; Deisseroth Albert B
AUTHOR ADDRESS: Sidney Kimmel Canc Ctr, Gene Therapy Program, San Diego, CA USA**USA
JOURNAL: Blood 106 (11, Part 2): p471B-472B NOV 16 2005 2005
CONFERENCE/MEETING: 47th Annual Meeting of the American-Society-of-Hematology Atlanta, GA, USA December 10 -13, 2005;

20051210
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

4/3/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18415151 BIOSIS NO.: 200510109651
Adenoviral vectors for targeting of cancer cells
AUTHOR: Deisseroth Albert (Reprint); Tang Yucheng; Liu Yanzheng; Akbulut Hakan; Maynard Jonathan; Zhang Lixin; Linton Phyllis- Jean
AUTHOR ADDRESS: Sidney Kimmel Canc Ctr, San Diego, CA USA**USA
JOURNAL: Cancer Gene Therapy 11 (12): p847 DEC 04 2004
CONFERENCE/MEETING: Meeting of the International-Society-for-Cancer-Gene-Therapy February 20 -22, 2004; 20040220
SPONSOR: Int Soc Cancer Gene Therapy
ISSN: 0929-1903
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

4/3/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17781412 BIOSIS NO.: 200400148073
An adenoviral vector cancer vaccine that delivers a tumor associated antigen/CD40-ligand fusion protein to dendritic cells in vivo and thereby breaks tolerance to tumor associated self antigens.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin (Reprint); Akbulut Hakan (Reprint); Litton Phyllis-Jean (Reprint); Deisseroth Albert B (Reprint)
AUTHOR ADDRESS: Genetic Therapy Program, Sidney Kimmel Cancer Center, San Diego, CA, USA**USA
JOURNAL: Blood 102 (11): p745a November 16, 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 45th Annual Meeting of the American Society of Hematology San Diego, CA, USA December 06-09, 2003; 20031206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

4/3/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17721687 BIOSIS NO.: 200400090456
An adenoviral vector cancer vaccine that delivers a tumor-associated antigen/CD40-ligand fusion protein to dendritic cells.

AUTHOR: Zhang Lixin; Tang Yucheng; Akbulut Hakan; Zelterman Daniel; Linton Phyllis-Jean; Deisseroth Albert B (Reprint)
AUTHOR ADDRESS: Sidney Kimmel Cancer Center, San Diego, CA, 92121, USA**USA
AUTHOR E-MAIL ADDRESS: adeisseroth@skcc.org
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 100 (25): p15101-15106 December 9, 2003 2003
MEDIUM: print
ISSN: 0027-8424 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

4/3/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17530335 BIOSIS NO.: 200300487992
Injection of adenoviral vector encoding a secretable form of the E7/CD40 ligand generates immunoresistance to E7 positive cell lines for over 1 year.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin (Reprint); Maynard Jonathan (Reprint); Deisseroth Albert (Reprint)
AUTHOR ADDRESS: Sidney Kimmel Cancer Center, San Diego, CA, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p589 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

4/3/9 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

17402648 PMID: 16928818
Antitumor immune response induced by i.t. injection of vector-activated dendritic cells and chemotherapy suppresses metastatic breast cancer.
Akbulut Hakan; Tang Yucheng; Akbulut K Gonca; Maynard Jonathan; Zhang Lixin; Deisseroth Albert
Sidney Kimmel Cancer Center, 10835 Road to the Cure, San Diego, CA 92121, USA.
Molecular cancer therapeutics (United States) Aug 2006, 5 (8) p1975-85, ISSN 1535-7163--Print 1535-7163--Linking Journal Code: 101132535
Publishing Model Print
Document type: Comparative Study; Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

4/3/10 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

16204250 PMID: 15238426
Multistep process through which adenoviral vector vaccine overcomes anergy to tumor-associated antigens.
Tang Yucheng; Zhang Lixin; Yuan Jing; Akbulut Hakan; Maynard Jonathan; Linton Phyllis-Jean; Deisseroth Albert
Sidney Kimmel Cancer Center, 10835 Altman Row, San Diego, CA 92121, USA.
Blood (United States) Nov 1 2004, 104 (9) p2704-13, ISSN 0006-4971 --Print 0006-4971--Linking Journal Code: 7603509
Publishing Model Print-Electronic
Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

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? s (adenoviral or adenovirus)(20n)(vector?)(20n)(Cd40L or cd154 or cd40(w)ligand)
          42047  ADENOVIRAL
         136081  ADENOVIRUS
         694836  VECTOR?
          10347  CD40L
           4611  CD154
          42860  CD40
         661511  LIGAND
          19956  CD40(W)LIGAND
      S5    379  (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?)(20N)(CD40L OR
                 CD154 OR CD40(W)LIGAND)
? s (adenoviral or adenovirus)(20n)(vector?)(20n)(Cd40L or cd154 or
cd40(w)ligand)(20n)(secret?)
          42047  ADENOVIRAL
         136081  ADENOVIRUS
         694836  VECTOR?
          10347  CD40L
           4611  CD154
          42860  CD40
         661511  LIGAND
          19956  CD40(W)LIGAND
        1567357  SECRET?
      S6     29  (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?)(20N)(CD40L OR
                 CD154 OR CD40(W)LIGAND)(20N)(SECRET?)
? rd s6
      S7     15  RD S6  (unique items)
? t s7/3/all
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7/3/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0021031254 BIOSIS NO.: 200900372691
Generation of Human Dendritic Cells That Simultaneously Secrete IL-12 and Have Migratory Capacity by Adenoviral Gene Transfer of hCD40L in Combination With IFN-gamma
AUTHOR: Knippertz Ilka; Hesse Andrea; Schunder Tania; Kaempgen Eckhart; Brenner Malcobn K; Schuler Gerold; Steinkasserer Alexander; Nettelbeck Dirk M (Reprint)
AUTHOR ADDRESS: Univ Heidelberg Hosp, German Canc Res Ctr, Helmholtz Univ Grp Oncolyt Adenoviruses, Neuenheimer Feld 242, D-69221 Heidelberg, Germany**Germany
AUTHOR E-MAIL ADDRESS: d.nettelbeck@dkfz-heidelberg.de
JOURNAL: Journal of Immunotherapy 32 (5): p524-538 JUN 2009 2009
ISSN: 1524-9557
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: English

7/3/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0020918470 BIOSIS NO.: 200900258804
Vaccination Strategies for Patients with B-CLLc
AUTHOR: Okur Fatma V (Reprint); Yvon Eric; Dotti Gianpietro; Carrum George; Heslop Helen E; Brenner Malcolm K; Fratantoni Joseph C; Peshwa Madhusudan V; Li Linhong
AUTHOR ADDRESS: Baylor Coll Med, Ctr Cell and Gene Therapy, Houston, TX 77030 USA**USA
JOURNAL: Blood 112 (11): p733 NOV 16 2008 2008
CONFERENCE/MEETING: 50th Annual Meeting of the American-Society-of-Hematology San Francisco, CA, USA December 06 -09, 2008; 20081206
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

7/3/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0020855343 BIOSIS NO.: 200900195677
CD40 ligation converts TGF-beta-secreting tolerogenic CD4(-)8(-) dendritic cells into IL-12-secreting immunogenic ones
AUTHOR: Zhang Xueshu; Kedl Ross M; Xiang Jim (Reprint)
AUTHOR ADDRESS: Univ Saskatchewan, Saskatchewan Canc Agcy, Canc Res Unit, 20 Campus Dr, Saskatoon, SK S7N 0W0, Canada**Canada
AUTHOR E-MAIL ADDRESS: Jim.Xiang@saskcancer.ca
JOURNAL: Biochemical and Biophysical Research Communications 379 (4): p 954-958 FEB 20 2009 2009
ITEM IDENTIFIER: doi:10.1016/j.bbrc.2008.12.179
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

7/3/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

19108867 BIOSIS NO.: 200600454262
Comparative analysis of antitumor activity of CD40L, RANKL, and 4-1BBL in vivo following intratumoral administration of viral vectors or transduced dendritic cells
AUTHOR: Yurkovetsky Zoya R; Shurin Galina V; Barry Denise A; Schuh Andre C; Shurin Michael R; Robbins Paul D (Reprint)
AUTHOR ADDRESS: Univ Pittsburgh, Sch Med, Dept Biochem and Mol Genet, W1246 Biomed Sci Tower, Pittsburgh, PA 15261 USA**USA
AUTHOR E-MAIL ADDRESS: probb@pitt.edu
JOURNAL: JOURNAL OF GENE MEDICINE 8 (2): p129-137 FEB 2006 2006
ISSN: 1099-498X_(print) 1521-2254_(electronic)
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: English

7/3/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18788546 BIOSIS NO.: 200600133941
Vector prime-protein boost vaccine induces immune response against "self-antigens" associated with epithelial neoplasms and tumor vascular endothelial cells.
AUTHOR: Tang Yucheng (Reprint); Maynard Jonathan; Akbulut Hakan; Linton Phyllis-Jean; Deisseroth Albert B
AUTHOR ADDRESS: Sidney Kimmel Canc Ctr, Gene Therapy Program, San Diego, CA USA**USA
JOURNAL: Blood 106 (11, Part 2): p471B-472B NOV 16 2005 2005
CONFERENCE/MEETING: 47th Annual Meeting of the American-Society-of-Hematology Atlanta, GA, USA December 10 -13, 2005; 20051210
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

7/3/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17781412 BIOSIS NO.: 200400148073
An adenoviral vector cancer vaccine that delivers a tumor associated antigen/CD40-ligand fusion protein to dendritic cells in vivo and thereby breaks tolerance to tumor associated self antigens.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin (Reprint); Akbulut Hakan (Reprint); Litton Phyllis-Jean (Reprint); Deisseroth Albert B (Reprint)
AUTHOR ADDRESS: Genetic Therapy Program, Sidney Kimmel Cancer Center, San Diego, CA, USA**USA
JOURNAL: Blood 102 (11): p745a November 16, 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 45th Annual Meeting of the American Society of Hematology San Diego, CA, USA December 06-09, 2003; 20031206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

7/3/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17649370 BIOSIS NO.: 200400016354
Enhanced effector and memory CTL responses generated by incorporation of receptor activator of NF-kappaB (RANK)/RANK ligand costimulatory molecules into dendritic cell immunogens expressing a human tumor-specific antigen.
AUTHOR: Wiethe Carsten; Dittmar Kurt; Doan Tracy; Lindenmaier Werner; Tindle Robert (Reprint)

AUTHOR ADDRESS: Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Herston Road, Herston, QLD, 4029, Australia** Australia
AUTHOR E-MAIL ADDRESS: r.tindle@mailbox.uq.edu.au
JOURNAL: Journal of Immunology 171 (8): p4121-4130 October 15, 2003 2003
MEDIUM: print
ISSN: 0022-1767 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

7/3/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17530335 BIOSIS NO.: 200300487992
Injection of adenoviral vector encoding a secretable form of the E7/CD40 ligand generates immunoresistance to E7 positive cell lines for over 1 year.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin (Reprint); Maynard Jonathan (Reprint); Deisseroth Albert (Reprint)
AUTHOR ADDRESS: Sidney Kimmel Cancer Center, San Diego, CA, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p589 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

7/3/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17398836 BIOSIS NO.: 200300357555
Treatment of High-Risk Acute Leukemia with an Autologous Vaccine Expressing Transgenic IL-2 and CD40L.
AUTHOR: Rousseau Raphael (Reprint); Biagi Ettore (Reprint); Yvon Eric (Reprint); Mei Zhuyong (Reprint); Inman Shannon (Reprint); Rill Donna (Reprint); Heslop Helen (Reprint); Popat Uday (Reprint); Gee Adrian (Reprint); Krance Robert (Reprint); Carrum George (Reprint); Alcoser Pat (Reprint); Rodgers Sherryl (Reprint); Kuehnle Ingrid (Reprint); Margolin Judith (Reprint); Brenner Malcolm (Reprint)
AUTHOR ADDRESS: Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA**USA
JOURNAL: Blood 100 (11): pAbstract No. 3420 November 16, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 44th Annual Meeting of the American Society of Hematology Philadelphia, PA, USA December 06-10, 2002; 20021206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

7/3/10 (Item 10 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

16593405 BIOSIS NO.: 200200186916
Membrane-stabilized chimeric tumor necrosis factor for gene therapy of B cell malignancies
AUTHOR: Cantwell Mark J (Reprint); Li Mei (Reprint); Prussak Charles (Reprint); Kipps Thomas J
AUTHOR ADDRESS: Tragen Pharmaceuticals, La Jolla, CA, USA**USA
JOURNAL: Blood 98 (11 Part 1): p423a November 16, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

7/3/11 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

16140629 BIOSIS NO.: 200100312468
Immune responses induced by autologous non-Hodgkin's lymphoma B cells expressing the CD40 ligand and interleukin-2 transgenes
AUTHOR: Takahashi Satoshi (Reprint); Rousseau Raphael F (Reprint); Yotnda Patricia (Reprint); Mei Zhuyong (Reprint); Smith Susan (Reprint); Donna Rill (Reprint); Brenner Malcolm K (Reprint)
AUTHOR ADDRESS: Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA**USA
JOURNAL: Blood 96 (11 Part 1): p340a November 16, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000; 20001201
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

7/3/12 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

15485486 BIOSIS NO.: 200000203799
Readministration of adenovirus vector in nonhuman primate lungs by blockade of CD40-CD40 ligand interactions
AUTHOR: Chirmule Narendra; Raper Steven E; Burkly Linda; Thomas David; Tazelaar John; Hughes Joseph V; Wilson James M (Reprint)
AUTHOR ADDRESS: University of Pennsylvania, 3601 Spruce St., 204 Wistar Institute, Philadelphia, PA, 19104, USA**USA
JOURNAL: Journal of Virology 74 (7): p3345-3352 April, 2000 2000
MEDIUM: print
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

7/3/13 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

0078161960 EMBASE/Medline No: 2000211248
CD40 ligand (CD154) enhances the Th1 and antibody responses to respiratory syncytial virus in the BALB/c mouse
Tripp R.A.; Jones L.; Anderson L.J.; Brown M.P.
Div. of Viral and Rickettsial Dis., Natl. Center of Infectious Diseases, Centers for Dis. Contr. and Prev., Atlanta, GA 30333, United States; Centers for Dis. Contr. and Prev., MS G09, 1600 Clifton Road, Atlanta, GA 30333, United States
AUTHOR EMAIL: rgt3@cdc.gov
CORRESP. AUTHOR/AFFIL: Tripp R.A.: Centers for Dis. Control/Prevention, 1600 Clifton Road, Atlanta, GA 30333, United States
CORRESP. AUTHOR EMAIL: rgt3@cdc.gov

Journal of Immunology (J. Immunol.) (United States) July 3, 2000, 164/11 (5913-5921)
CODEN: JOIMA ISSN: 0022-1767
DOCUMENT TYPE: Journal; Article RECORD TYPE: Abstract
LANGUAGE: English SUMMARY LANGUAGE: English
NUMBER OF REFERENCES: 71

7/3/14 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

0069486677 EMBASE/Medline No: 16256021
Construction of recombinant adenovirus expressing sCD40L-Ig
Li Z.L.; Tian P.X.; Xue W.J.
Renal Disease Center of First Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710061, China.
CORRESP. AUTHOR/AFFIL: Li Z.L.: Renal Disease Center of First Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710061, China.
CORRESP. AUTHOR EMAIL: Lizhaolun1@sina.com.cn

Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology (Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi) (China) November 1, 2005, 21/6 (668-671)
ISSN: 1007-8738
DOCUMENT TYPE: Journal; Article RECORD TYPE: Abstract
FILE SEGMENT: Medline
LANGUAGE: Chinese

7/3/15 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

32969660 PMID: 20423644
[Construction and identification of recombinant adenovirus vector expressing IkappaBalpha-IRES2-shCD40L.]
Ding Xiao-Ming; Niu Xiao-Li; Xue Wu-Jun; Li Yang
Department of Renal Transplantation, Center of Nephropathy, First Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710061, China.
Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology (China) May 2010, 26 (5) p416-9, ISSN 1007-8738 --Print 1007-8738--Linking Journal Code: 101139110

Publishing Model Print
Document type: English Abstract; Journal Article
Languages: CHINESE
Main Citation Owner: NLM
Record type: In Data Review
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7/7/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17398836 BIOSIS NO.: 200300357555
Treatment of High-Risk Acute Leukemia with an Autologous Vaccine Expressing Transgenic IL-2 and CD40L.
AUTHOR: Rousseau Raphael (Reprint); Biagi Ettore (Reprint); Yvon Eric (Reprint); Mei Zhuyong (Reprint); Inman Shannon (Reprint); Rill Donna (Reprint); Heslop Helen (Reprint); Popat Uday (Reprint); Gee Adrian (Reprint); Krance Robert (Reprint); Carrum George (Reprint); Alcoser Pat (Reprint); Rodgers Sherryl (Reprint); Kuehnle Ingrid (Reprint); Margolin Judith (Reprint); Brenner Malcolm (Reprint)
AUTHOR ADDRESS: Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA**USA
JOURNAL: Blood 100 (11): pAbstract No. 3420 November 16, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 44th Annual Meeting of the American Society of Hematology Philadelphia, PA, USA December 06-10, 2002; 20021206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English
ABSTRACT: Leukemic cells generally do not express the costimulatory surface molecules necessary for induction of a T-cell response. Consequently, they induce specific T-cell anergy. Engagement of CD40L augments antigen presentation by normal and malignant B cells, and by antigen-presenting cells (APC) by up-regulating the expression of adhesion, costimulatory and MHC molecules. Stimulation of APC through the CD40-CD40L pathway bypasses the helper T-cell mechanism in activating specific cytotoxic T cells. In murine models, CD40L augments the immune response against CD40-malignancies by stimulating activated CD4+ and CD8+ T cells. Hence, activation of CD40+ leukemia cells by CD40L generates an anti-tumor response in leukemia-bearing mice and the effect is potentiated by IL2 (Dilloo et al., Blood 1997;90:1927). We developed a Phase I study to assess the feasibility, safety and immunologic efficacy of an IL2- and CD40L-expressing tumor vaccine in patients with high-risk acute leukemia. The predicted relapse risk for this group was >50% at 2 years. Autologous skin fibroblasts were transduced with adenoviral ***vectors*** encoding human IL-2 and ***CD40L*** . High-risk patients in complete or partial cytological remission received up to six s.c.injections of their gene-modified ***CD40L*** and IL-2 fibroblasts, and leukemic blasts, separated by one-two weeks in the absence of concurrent therapy. Patients received a fixed dose of IL-2 secreting fibroblasts (2x1E7 per injection) and leukemic blasts (2x1E7 per injection) throughout the treatment protocol, while the dose of CD40L-secreting fibroblasts were escalated from 2x1E5 (level 1) to 2x1E7 (level 3) per injection. To date, nine patients (2 adults, 7 children) with AML (3 patients) or B-ALL (5 patients) have been studied. All but 1 patient were in complete remission on enrollment, 7 post allogeneic bone marrow transplantation and 1 post chemotherapy regimen. All patients were off immunosuppressive drugs. No severe adverse reactions were noted. Of the 8 evaluable patients, one relapsed (skull

infiltrate) after 22 weeks. All other patients remain disease free 1 to 31 months after the 1st injection (disease free survival at 12 months = 87.5%). Injection-site biopsies revealed increased cellularity due to infiltration of CD3+ cells. Systemically, we observed a significant expansion of the CD4+ (259+- 35/ml to 519+-66/ml, a 2-fold increase, P=0.004) and the CD3+CD25+ T-cell (100+-20/ml to 189+-11/ml, a 1.9-fold increase, P=0.006) populations. Using the ELISPOT assay, we found an increase in IFNgamma- and IL4-spot forming cells reactive to their autologous blasts after 3 injections (IFNgamma: median 10 pre to 190 post: IL-4; median 10 pre to 40 post). Two of 8 evaluable patients produced IgG antibodies that bound to their autologous blasts. Thus, a vaccine combining transgenic skin fibroblasts secreting CD40L with IL-2 and autologous leukemic blasts can be safely administered to patients in remission of acute leukemia, and even in patients post allogeneic bone marrow transplantation, it can produce immunomodulation. A larger study with continued follow up should indicate whether such adjuvant therapy has clinical benefit.

7/7/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

16593405 BIOSIS NO.: 200200186916
Membrane-stabilized chimeric tumor necrosis factor for gene therapy of B cell malignancies
AUTHOR: Cantwell Mark J (Reprint); Li Mei (Reprint); Prussak Charles (Reprint); Kipps Thomas J
AUTHOR ADDRESS: Tragen Pharmaceuticals, La Jolla, CA, USA**USA
JOURNAL: Blood 98 (11 Part 1): p423a November 16, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Tumor necrosis factor (TNF) first was identified as a molecule that could induce apoptosis (previously considered necrosis) of tumor cells when injected into tumor-bearing animals. Clinical trials in patients with various cancers, however, revealed TNF had a low therapeutic index, in part due to the high systemic toxicity of soluble TNF, thereby greatly limiting the concentration of TNF that could be achieved at sites of tumor in vivo. Nevertheless, encouraging clinical responses were observed, particularly in patients with B cell malignancies (Selby et. al., Br J Cancer. 56:803-808, 1987). Transduction of tumor cells with genes encoding TNF might be an effective strategy for treatment of such neoplastic diseases. However, this strategy may also generate unacceptable toxicities, as the membrane-associated pro-cytokine of wild-type TNF (wtTNF) is readily cleaved, releasing a soluble cytokine that diffuses rapidly to distal sites in vivo. Transfer of genes encoding membrane-stabilized forms of TNF, on the other hand, may allow for high-level local expression of molecules that can effect TNF-signaling without the systemic toxicity associated with soluble TNF. To this end, we generated chimeric TNF genes encoding the receptor-binding domain of TNF spliced onto transmembrane domains of other members of the TNF family (e.g. CD70, ***CD154*** , TRAIL, and Fas-Ligand). In addition, we introduced an in-frame deletion to generate a truncated TNF gene (DELTATNF) lacking the known site(s) for cleavage by matrix

metalloproteinases. Finally, we generated recombinant ***adenovirus*** (Ad) ***vectors*** encoding these recombinant TNF genes. These Ad vectors were used to transduce cells that subsequently were examined for expression of soluble and membrane-anchored molecules with TNF activity. We discovered that cells transduced with Ad encoding the chimeric CD154-TNF (Ad-CD154-TNF) expressed significantly higher levels of cell-surface TNF than did cells equally transduced with Ad-wtTNF, Ad-DELTATNF, or any one of the other chimeric constructs. Moreover, cells that expressed CD154-TNF specifically could interact with both p55 and p75 TNF-receptors (CD120a and CD120b) to effect TNF-signaling. On the other hand, cells expressing the chimeric CD154-TNF gene secreted near-negligible amounts of soluble TNF that were 1/1,000th or 1/10Qth of that produced by equivalent numbers of cells transduced with Ad-wtTNF or Ad-DELTATNF, respectively. Neoplastic B cells from patients with chronic lymphocytic leukemia, follicular lymphoma, or multiple myeloma also could be transduced with Ad-CD154-TNF. Transduced B cells expressed high surface levels of TNF without releasing detectable amounts of soluble TNF. Furthermore, transduction of the neoplastic B cells with Ad-CD154-TNF induced expression of immune co-stimulatory molecules that are important for antigen presentation. We conclude that CD154-TNF represents a novel type of membrane-stabilized TNF that has potent biologic activity. The use of such molecules could mitigate the risk of systemic toxicity caused by soluble TNF, potentially allowing for the application of TNF gene therapy in patients with B cell malignancies.

7/7/11 (Item 11 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

16140629 BIOSIS NO.: 200100312468
Immune responses induced by autologous non-Hodgkin's lymphoma B cells expressing the CD40 ligand and interleukin-2 transgenes
AUTHOR: Takahashi Satoshi (Reprint); Rousseau Raphael F (Reprint); Yotnda Patricia (Reprint); Mei Zhuyong (Reprint); Smith Susan (Reprint); Donna Rill (Reprint); Brenner Malcolm K (Reprint)
AUTHOR ADDRESS: Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA**USA
JOURNAL: Blood 96 (11 Part 1): p340a November 16, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000; 20001201
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The malignant B cells of non-Hodgkin's lymphoma (B-NHL) express peptides derived from tumor specific antigens (such as immunoglobulin idiotypes), and also express major histocompatibility complex (MHC) antigens. However, they do not express co-stimulatory molecules which likely contributes to their protection from host antitumor immunity. To stimulate NHL-specific immune responses, we attempted to transfer the human CD40 ligand (hCD40L) gene to B-NHL cells and enhance their co-stimulatory potential. We found an ***adenoviral*** ***vector*** encoding human CD40L (AdhCD40L) was ineffective at transducing B-NHL cells, which lack adenoviral receptors, including CAR (the coxsackievirus B- ***adenovirus*** receptors) and alphav integrins. However, pre-culture of the B-NHL cells with human embryonic lung fibroblast line MRC-5 significantly upregulated expression of integrins

and markedly increased their susceptibility to adenoviral ***vector*** transduction. After pre-stimulation, transduction with AdhCD40L increased ***CD40L*** expression on B-NHL cells from 1.3+-0.2% to 40.8+-11.9% (n=7). No significant increase in ***CD40L*** expression was obtained without pre-culture or with control advectors. Expression of transgenic human CD40L was in turn associated with upregulation of other co-stimulatory molecules including B7-1/-2 (CD80 expression before transduction: 12.2+-6.3%; after transduction: 46.0+-10.4%; CD86 expression before transduction: 42.9+-6.2%; after transduction: 71.3+-7.9%). Transduced B-NHL cells were now able to stimulate autologous T cells to proliferate and secrete Th1 cytokines, but the stimulated T cells were unable to recognize unmodified lymphoma cells - a requirement for an effective tumor vaccine. Our previous studies of murine lymphoma models suggested that CD40L and interleukin-2 (IL2) in combination were more potent than either molecule alone. We therefore transduced B-NHL cells with AdhCD40L and AdhIL2 (IL2 production before transduction: below limit of detection; on day 3: 10.1+-5.2ng of IL2/106 cells/24 hours). Although IL2 transduction alone had little effect, admixture of hCD40L- and hIL2-gene transduced cells enhanced initial T-cell activation and also generated autologous T cells capable of specifically recognizing and killing parental B-NHL cells via MHC restricted cytotoxic T lymphocytes. These findings suggest that the combination of CD40L and IL2 gene-modified B-NHL cells may be capable of inducing a cytotoxic immune response in vivo.

7/7/12 (Item 12 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

15485486 BIOSIS NO.: 200000203799
Readministration of adenovirus vector in nonhuman primate lungs by blockade of CD40-CD40 ligand interactions
AUTHOR: Chirmule Narendra; Raper Steven E; Burkly Linda; Thomas David; Tazelaar John; Hughes Joseph V; Wilson James M (Reprint)
AUTHOR ADDRESS: University of Pennsylvania, 3601 Spruce St., 204 Wistar Institute, Philadelphia, PA, 19104, USA**USA
JOURNAL: Journal of Virology 74 (7): p3345-3352 April, 2000 2000
MEDIUM: print
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The interaction between CD40 on B cells and CD40 ligand (CD40L) on activated T cells is important for B-cell differentiation in T-cell-dependent humoral responses. We have extended our previous murine studies of CD40-CD40L in adenoviral ***vector*** -mediated immune responses to rhesus monkeys. Primary immune responses to adenoviral vectors and the ability to readminister vector were studied in rhesus monkeys in the presence or absence of a transient treatment with a humanized anti-CD40 ***ligand*** antibody (hu5C8). Adult animals were treated with hu5C8 at the time ***vector*** was instilled into the lung. Immunological analyses demonstrated suppression of adenovirus-induced lymphoproliferation and cytokine responses (interleukin-2 (IL-2), gamma interferon, IL-4, and IL-10) in hu5C8-treated animals. Animals treated with hu5C8 secreted adenovirus-specific immunoglobulin M (IgM) levels comparable to control animals, but did not secrete IgA or develop neutralizing antibodies; consequently, the animals could be readministered with adenovirus vector expressing alkaline

phosphatase. A second study was designed to examine the long-term effects on immune functions of a short course of hu5C8. Acute hu5C8 treatment resulted in significant and prolonged inhibition of the adenovirus-specific humoral response well beyond the time hu5C8 effects were no longer significant. These studies demonstrate the potential of hu5C8 as an immunomodulatory regimen to enable administration of adenoviral vectors, and they advocate testing this model in humans.

7/7/13 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

0078161960 EMBASE/Medline No: 2000211248
CD40 ligand (CD154) enhances the Th1 and antibody responses to respiratory syncytial virus in the BALB/c mouse
Tripp R.A.; Jones L.; Anderson L.J.; Brown M.P.
Div. of Viral and Rickettsial Dis., Natl. Center of Infectious Diseases, Centers for Dis. Contr. and Prev., Atlanta, GA 30333, United States; Centers for Dis. Contr. and Prev., MS G09, 1600 Clifton Road, Atlanta, GA 30333, United States
AUTHOR EMAIL: rgt3@cdc.gov
CORRESP. AUTHOR/AFFIL: Tripp R.A.: Centers for Dis. Control/Prevention, 1600 Clifton Road, Atlanta, GA 30333, United States
CORRESP. AUTHOR EMAIL: rgt3@cdc.gov

Journal of Immunology (J. Immunol.) (United States) July 3, 2000, 164/11 (5913-5921)
CODEN: JOIMA ISSN: 0022-1767
DOCUMENT TYPE: Journal; Article RECORD TYPE: Abstract
LANGUAGE: English SUMMARY LANGUAGE: English
NUMBER OF REFERENCES: 71

CD40 ligand (CD40L) is a cell surface costimulatory molecule expressed mainly by activated T cells. CD40L is critically important for T-B cell and T cell-dendritic cell interactions. CD40L expression promotes Th1 cytokine responses to protein Ags and is responsible for Ig isotype switching in B cells. Respiratory syncytial virus (RSV) is an important pathogen of young children and the elderly, which causes bronchiolitis and pneumonia. Studies of mice infected with RSV suggest that a Th2 cytokine response may be responsible for enhanced pulmonary disease. To investigate the effect CD40L has on RSV immunity, mice were infected simultaneously with RSV and either an empty control adenovirus vector or one expressing CD40L or were coimmunized with plasmid DNA vectors expressing CD40L and RSV F and/or G proteins and subsequently challenged with RSV. The kinetics of the intracellular and ***secreted*** cytokine responses, the cytotoxic T lymphocyte precursor frequency, NO levels in lung lavage, rates of virus clearance, and anti-RSV Ab titers were determined. These studies show that coincident expression of CD40L enhances the Th1 (IL-2 and IFN-gamma) cytokine responses, increases the expression of TNF-alpha and NO, accelerates virus clearance, and increases the anti-F and anti-G Ab responses. These data suggest that CD40L may have the adjuvant properties needed to optimize the safety and efficacy of RSV vaccines.
? ds

Set	Items	Description
S1	166	E1-E4
S2	269	AU='ZHANG LIXIN'
S3	12	(S1 OR S2) AND (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?) AND (CD40L OR CD154 OR CD40(W)LIGAND)

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S4          10   RD S3  (unique items)
S5         379   (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?)(20N)(CD40L OR CD1-
              54 OR CD40(W)LIGAND)
S6          29   (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?)(20N)(CD40L OR CD1-
              54 OR CD40(W)LIGAND)(20N)(SECRET?)
S7          15   RD S6  (unique items)
? s (adenoviral or adenovirus)(20n)(vector?)(20n)(Cd40L or cd154 or cd40(w)ligand)
and (cd40L or cd40(w)ligand or cd154)(20n)(secret?)
           42047  ADENOVIRAL
          136081  ADENOVIRUS
          694836  VECTOR?
           10347  CD40L
            4611  CD154
           42860  CD40
          661511  LIGAND
           19956  CD40(W)LIGAND
             384  (ADENOVIRAL OR ADENOVIRUS)(20N)VECTOR?(20N)((CD40L OR
                  CD154) OR CD40(W)LIGAND)
           10347  CD40L
           42860  CD40
          661511  LIGAND
           19956  CD40(W)LIGAND
            4611  CD154
         1567357  SECRET?
            1381  ((CD40L OR CD40(W)LIGAND) OR CD154)(20N)SECRET?
      S8      28  (ADENOVIRAL OR ADENOVIRUS)(20N)(VECTOR?)(20N)(CD40L OR
                  CD154 OR CD40(W)LIGAND) AND (CD40L OR CD40(W)LIGAND OR
                  CD154)(20N)(SECRET?)
? rd s8
      S9      19  RD S8  (unique items)
? t s9/3/all

 9/3/1     (Item 1 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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0021031254   BIOSIS NO.: 200900372691
Generation of Human Dendritic Cells That Simultaneously Secrete IL-12 and
  Have Migratory Capacity by Adenoviral Gene Transfer of hCD40L in
  Combination With IFN-gamma
AUTHOR: Knippertz Ilka; Hesse Andrea; Schunder Tania; Kaempgen Eckhart;
  Brenner Malcobn K; Schuler Gerold; Steinkasserer Alexander; Nettelbeck
  Dirk M (Reprint)
AUTHOR ADDRESS: Univ Heidelberg Hosp, German Canc Res Ctr, Helmholtz Univ
  Grp Oncolyt Adenoviruses, Neuenheimer Feld 242, D-69221 Heidelberg,
  Germany**Germany
AUTHOR E-MAIL ADDRESS: d.nettelbeck@dkfz-heidelberg.de
JOURNAL: Journal of Immunotherapy  32 (5): p524-538 JUN 2009 2009
ISSN: 1524-9557
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English


 9/3/2     (Item 2 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)
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0020918470   BIOSIS NO.: 200900258804
Vaccination Strategies for Patients with B-CLLc
AUTHOR: Okur Fatma V (Reprint); Yvon Eric; Dotti Gianpietro; Carrum George;
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Heslop Helen E; Brenner Malcolm K; Fratantoni Joseph C; Peshwa Madhusudan V; Li Linhong
AUTHOR ADDRESS: Baylor Coll Med, Ctr Cell and Gene Therapy, Houston, TX 77030 USA**USA
JOURNAL: Blood 112 (11): p733 NOV 16 2008 2008
CONFERENCE/MEETING: 50th Annual Meeting of the American-Society-of-Hematology San Francisco, CA, USA December 06 -09, 2008; 20081206
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

9/3/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

19108867 BIOSIS NO.: 200600454262
Comparative analysis of antitumor activity of CD40L, RANKL, and 4-1BBL in vivo following intratumoral administration of viral vectors or transduced dendritic cells
AUTHOR: Yurkovetsky Zoya R; Shurin Galina V; Barry Denise A; Schuh Andre C; Shurin Michael R; Robbins Paul D (Reprint)
AUTHOR ADDRESS: Univ Pittsburgh, Sch Med, Dept Biochem and Mol Genet, W1246 Biomed Sci Tower, Pittsburgh, PA 15261 USA**USA
AUTHOR E-MAIL ADDRESS: probb@pitt.edu
JOURNAL: JOURNAL OF GENE MEDICINE 8 (2): p129-137 FEB 2006 2006
ISSN: 1099-498X_(print) 1521-2254_(electronic)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

9/3/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18573019 BIOSIS NO.: 200510267519
Soluble factors secreted from CD40 ligand-transfected dendritic cells enhance TRAIL-induced apoptosis of multiple myeloma.
AUTHOR: Tomihara Kei (Reprint); Kato Kazunori; Hamada Hirofumi
AUTHOR ADDRESS: Sapporo Med Univ, Dept Mol Med, Sapporo, Hokkaido, Japan** Japan
JOURNAL: Blood 104 (11, Part 2): p300B NOV 16 2004 2004
CONFERENCE/MEETING: 46th Annual Meeting of the American-Society-of-Hematology San Diego, CA, USA December 04 -07, 2004; 20041204
SPONSOR: Amer Soc Hematol
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

9/3/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17781412 BIOSIS NO.: 200400148073

An adenoviral vector cancer vaccine that delivers a tumor associated antigen/CD40-ligand fusion protein to dendritic cells in vivo and thereby breaks tolerance to tumor associated self antigens.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin (Reprint); Akbulut Hakan (Reprint); Litton Phyllis-Jean (Reprint); Deisseroth Albert B (Reprint)
AUTHOR ADDRESS: Genetic Therapy Program, Sidney Kimmel Cancer Center, San Diego, CA, USA**USA
JOURNAL: Blood 102 (11): p745a November 16, 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 45th Annual Meeting of the American Society of Hematology San Diego, CA, USA December 06-09, 2003; 20031206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Poster; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

9/3/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17649370 BIOSIS NO.: 200400016354
Enhanced effector and memory CTL responses generated by incorporation of receptor activator of NF-kappaB (RANK)/RANK ligand costimulatory molecules into dendritic cell immunogens expressing a human tumor-specific antigen.
AUTHOR: Wiethe Carsten; Dittmar Kurt; Doan Tracy; Lindenmaier Werner; Tindle Robert (Reprint)
AUTHOR ADDRESS: Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Herston Road, Herston, QLD, 4029, Australia** Australia
AUTHOR E-MAIL ADDRESS: r.tindle@mailbox.uq.edu.au
JOURNAL: Journal of Immunology 171 (8): p4121-4130 October 15, 2003 2003
MEDIUM: print
ISSN: 0022-1767 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

9/3/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17530335 BIOSIS NO.: 200300487992
Injection of adenoviral vector encoding a secretable form of the E7/CD40 ligand generates immunoresistance to E7 positive cell lines for over 1 year.
AUTHOR: Tang Yucheng (Reprint); Zhang Lixin (Reprint); Maynard Jonathan (Reprint); Deisseroth Albert (Reprint)
AUTHOR ADDRESS: Sidney Kimmel Cancer Center, San Diego, CA, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p589 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation

LANGUAGE: English

9/3/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17398836 BIOSIS NO.: 200300357555
Treatment of High-Risk Acute Leukemia with an Autologous Vaccine Expressing Transgenic IL-2 and CD40L.
AUTHOR: Rousseau Raphael (Reprint); Biagi Ettore (Reprint); Yvon Eric (Reprint); Mei Zhuyong (Reprint); Inman Shannon (Reprint); Rill Donna (Reprint); Heslop Helen (Reprint); Popat Uday (Reprint); Gee Adrian (Reprint); Krance Robert (Reprint); Carrum George (Reprint); Alcoser Pat (Reprint); Rodgers Sherryl (Reprint); Kuehnle Ingrid (Reprint); Margolin Judith (Reprint); Brenner Malcolm (Reprint)
AUTHOR ADDRESS: Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX, USA**USA
JOURNAL: Blood 100 (11): pAbstract No. 3420 November 16, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 44th Annual Meeting of the American Society of Hematology Philadelphia, PA, USA December 06-10, 2002; 20021206
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

9/3/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

16626659 BIOSIS NO.: 200200220170
Comparing the efficiency of adenoviral gene transfer of CD40-ligand (CD154) versus treatment with immunostimulatory DNA-sequences as cellular anti-lymphoma vaccines in the murine A20 model
AUTHOR: Rieger Roman (Reprint); Kipps Thomas J (Reprint)
AUTHOR ADDRESS: School of Medicine, Division of Hematology/Oncology, University of California, La Jolla, CA, USA**USA
JOURNAL: Blood 98 (11 Part 1): p609a November 16, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

9/3/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

16593405 BIOSIS NO.: 200200186916
Membrane-stabilized chimeric tumor necrosis factor for gene therapy of B cell malignancies
AUTHOR: Cantwell Mark J (Reprint); Li Mei (Reprint); Prussak Charles (Reprint); Kipps Thomas J
AUTHOR ADDRESS: Tragen Pharmaceuticals, La Jolla, CA, USA**USA

JOURNAL: Blood 98 (11 Part 1): p423a November 16, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207
SPONSOR: American Society of Hematology
ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract; Meeting Poster
RECORD TYPE: Abstract
LANGUAGE: English

9/3/11 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

0080486566 EMBASE/Medline No: 2005130724
Molecular transfer of CD40 and OX40 ligands to leukemic human B cells induces expansion of autologous tumor-reactive cytotoxic T lymphocytes
Biagi E.; Dotti G.; Yvon E.; Lee E.; Pule M.; Vigouroux S.; Gottschalk S.; Popat U.; Rousseau R.; Brenner M.
Center for Cell and Gene Therapy, Baylor College of Medicine, Methodist Hosp./Texas Children's H., Houston, TX, United States; Center for Cell and Gene Therapy, 1102 Bates St., Houston, TX 77030, United States
AUTHOR EMAIL: exbiagi@txccc.org
CORRESP. AUTHOR/AFFIL: Biagi E.: Center for Cell and Gene Therapy, 1102 Bates St., Houston, TX 77030, United States
CORRESP. AUTHOR EMAIL: exbiagi@txccc.org

Blood (Blood) (United States) March 15, 2005, 105/6 (2436-2442)
CODEN: BLOOA ISSN: 0006-4971
DOI: 10.1182/blood-2004-07-2556
DOCUMENT TYPE: Journal; Article RECORD TYPE: Abstract
LANGUAGE: English SUMMARY LANGUAGE: English
NUMBER OF REFERENCES: 38

9/3/12 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

0078161960 EMBASE/Medline No: 2000211248
CD40 ligand (CD154) enhances the Th1 and antibody responses to respiratory syncytial virus in the BALB/c mouse
Tripp R.A.; Jones L.; Anderson L.J.; Brown M.P.
Div. of Viral and Rickettsial Dis., Natl. Center of Infectious Diseases, Centers for Dis. Contr. and Prev., Atlanta, GA 30333, United States; Centers for Dis. Contr. and Prev., MS G09, 1600 Clifton Road, Atlanta, GA 30333, United States
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0069486677 EMBASE/Medline No: 16256021
Construction of recombinant adenovirus expressing sCD40L-Ig
Li Z.L.; Tian P.X.; Xue W.J.
Renal Disease Center of First Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710061, China.
CORRESP. AUTHOR/AFFIL: Li Z.L.: Renal Disease Center of First Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710061, China.
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Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology (Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi) (China)
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FILE SEGMENT: Medline
LANGUAGE: Chinese

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DIALOG(R)File 155:MEDLINE(R)

32969660 PMID: 20423644
[Construction and identification of recombinant adenovirus vector expressing IkappaBalpha-IRES2-shCD40L.]
Ding Xiao-Ming; Niu Xiao-Li; Xue Wu-Jun; Li Yang
Department of Renal Transplantation, Center of Nephropathy, First Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710061, China.
Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology (China) May 2010, 26 (5) p416-9, ISSN 1007-8738 --Print 1007-8738--Linking Journal Code: 101139110
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9/3/15 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

147008378 CA: 147(1)8378d PATENT
Fusion proteins comprising CD40 ligand and pathogen or tumor antigen as vaccines against infection or cancer
INVENTOR(AUTHOR): Tang, Yucheng; Deisseroth, Albert
LOCATION: USA
ASSIGNEE: Sidney Kimmel Cancer Center
PATENT: PCT International ; WO 200756266 A2 DATE: 20070518
APPLICATION: WO 2006US43164 (20061106) *US 2005PV734136 (20051107) *US 2006PV755885 (20060104) *US 2006PV789270 (20060404) *US 2006PV793206 (20060419) *US 2006PV853184 (20061020)
PAGES: 122pp. CODEN: PIXXD2 LANGUAGE: English
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A61K-0039/145 A I F B 20060101 H US
A61K-0048/00 A I L B 20060101 H US

DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BW; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; EG; ES; FI; GB; GD; GE; GH; GM; GT; HN; HR; HU; ID; IL; IN; IS; JP; KE; KG; KM; KN; KP; KR; KZ; LA; LC; LK; LR; LS; LT; LU; LV; LY; MA; MD; MG; MK; MN; MW; MX; MY; MZ; NA; NG; NI; NO; NZ; OM; PG; PH; PL; PT; RO; RS; RU; SC; SD; SE; SG; SK; SL; SM; SV; SY; TJ; TM; TN; TR; TT; TZ; UA; UG DESIGNATED REGIONAL: AT; BE; BG; CH ; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR; HU; IE; IS; IT; LT; LU; LV; MC; NL; PL; PT; RO; SE; SI; SK; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG; BW; GH; GM; KE; LS; MW; MZ; NA; SD; SL; SZ; TZ; UG; ZM; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM

9/3/16 (Item 2 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

146054910 CA: 146(4)54910y JOURNAL
Chemotherapeutic agents enhance AAV2-mediated gene transfer into breast cancer cells promoting CD40 ligand-based immunotherapy
AUTHOR(S): Koppold, Bernd; Sauer, Georg; Buening, Hildegard; Hallek, Michael; Kreienberg, Rolf; Deissler, Helmut; Kurzeder, Christian
LOCATION: Department of Obstetrics and Gynecology, University of Ulm Medical School, Ulm, Germany, 89075
JOURNAL: J. Cancer Res. Clin. Oncol. (Journal of Cancer Research and Clinical Oncology) DATE: 2006 VOLUME: 132 NUMBER: 12 PAGES: 787-794
CODEN: JCROD7 ISSN: 0171-5216 LANGUAGE: English PUBLISHER: Springer

9/3/17 (Item 3 from file: 399)
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146044177 CA: 146(3)44177a PATENT
Methods for immunotherapy of cancer using an expression vector encoding a tumor vasculature antigen (TVECA)-CD40L fusion and/or a tumor antigen vaccine
INVENTOR(AUTHOR): Tang, Yucheng; Deisseroth, Albert
LOCATION: USA
ASSIGNEE: Sidney Kimmel Cancer Center
PATENT: PCT International ; WO 2006130525 A2 DATE: 20061207
APPLICATION: WO 2006US20652 (20060526) *US 2005PV686534 (20050531) *US 2006PV795686 (20060428)
PAGES: 80pp. CODEN: PIXXD2 LANGUAGE: English
PATENT CLASSIFICATIONS:
CLASS: A61K-000/A
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BW; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; EG; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KM; KN; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; LY; MA; MD; MG; MK; MN; MW; MX; MZ; NA; NG; NI; NO; NZ; OM; PG; PH; PL; PT; RO; RU; SC; SD; SE; SG; SK; SL; SM; SY; TJ; TM; TN; TR; TT; TZ; UA; UG; US; UZ; VC; VN; YU; ZA DESIGNATED REGIONAL: AT; BE; BG; CH ; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR; HU; IE; IS; IT; LT; LU; LV; MC; NL; PL; PT; RO; SE; SI; SK; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG; BW; GH; GM; KE; LS; MW; MZ; NA; SD; SL; SZ; TZ; UG; ZM; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM

9/3/18 (Item 4 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

143095805 CA: 143(6)95805z PATENT
Vectors encoding antigen-CD40 ligand fusion proteins for generating immunity against cancerous and infectious diseases
INVENTOR(AUTHOR): Diesseroth, Albert; Tang, Yucheng; Zhang, Wei-Wei; Fang, Xiang-Ming
LOCATION: USA
ASSIGNEE: Sidney Kimmel Cancer Center
PATENT: PCT International ; WO 200558950 A2 DATE: 20050630
APPLICATION: WO 2004US41690 (20041210) *US 2003PV592016 (20031211)
PAGES: 65 pp. CODEN: PIXXD2 LANGUAGE: English
PATENT CLASSIFICATIONS:
CLASS: C07K-014/47A
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BW; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; EG; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NA; NI; NO; NZ; OM; PG; PH; PL; PT; RO; RU; SC; SD; SE; SG; SK; SL; SY; TJ; TM; TN; TR; TT; TZ; UA; UG; US; UZ; VC; VN; YU; ZA; ZM; ZW DESIGNATED REGIONAL: BW; GH; GM; KE; LS; MW; MZ ; NA; SD; SL; SZ; TZ; UG; ZM; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM; AT; BE; BG; CH; CY; CZ; DE; DK; EE; ES; FI; FR; GB; GR; HU; IE; IS; IT; LT; LU; MC; NL; PL; PT; RO; SE; SI; SK; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG

9/3/19 (Item 5 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

132320690 CA: 132(24)320690c JOURNAL
Readministration of adenovirus vector in nonhuman primate lungs by blockade of CD40-CD40 ligand interactions
AUTHOR(S): Chirmule, Narendra; Raper, Steven E.; Burkly, Linda; Thomas, David; Tazelaar, John; Hughes, Joseph V.; Wilson, James M.
LOCATION: Institute for Human Gene Therapy, Department of Molecular and Cellular Engineering, University of Pennsylvania, Philadelphia, PA, USA
JOURNAL: J. Virol. DATE: 2000 VOLUME: 74 NUMBER: 7 PAGES: 3345-3352
CODEN: JOVIAM ISSN: 0022-538X LANGUAGE: English PUBLISHER: American Society for Microbiology
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9/7/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

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Comparing the efficiency of adenoviral gene transfer of CD40-ligand (CD154) versus treatment with immunostimulatory DNA-sequences as cellular anti-lymphoma vaccines in the murine A20 model
AUTHOR: Rieger Roman (Reprint); Kipps Thomas J (Reprint)
AUTHOR ADDRESS: School of Medicine, Division of Hematology/Oncology, University of California, La Jolla, CA, USA**USA
JOURNAL: Blood 98 (11 Part 1): p609a November 16, 2001 2001
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SPONSOR: American Society of Hematology
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The interaction between CD40 on antigen-presenting cells like B cells and its ligand CD40L (CD154) on activated T cells plays a critical role in the initiation of immune responses, including anti-tumor immunity. This interaction induces B cells to express co-stimulatory molecules, such as CD80 (B7-1), which are necessary for efficient antigen presentation. Most B cell leukemias and lymphomas also express CD40 and are induced to express co-stimulatory molecules upon exposure to CD154-bearing cells. A20 is a BALB/c-derived B cell lymphoma line that has many features in common with human B cell neoplasms. A20 cells express B cell differentiation antigens, CD40, and high-levels of class I and class II major histocompatibility complex (MHC) antigens. Despite expression of MHC antigens required for T cell antigen presentation, A20 cells are poor antigen presenting cells (APC) and cannot stimulate significant autologous, or even allogeneic, mixed lymphocyte reactions (MLR). ***Adenovirus*** (Ad)- ***vector*** gene transfer of murine CD40-ligand (CD154) into A20 cells results in high-level expression of CD154, which ligates CD40 on both infected and non-infected bystander A20 cells. This induces A20 cells to express immune co-stimulatory molecules, such as CD80 (B7-1), that are essential for effective APC activity. Immunostimulatory DNA sequences (ISS) containing non-methylated CpG dinucleotides within a defined motif also can induce such changes in A20 cells. We examined the antigen-presenting activity of A20 cells that were infected with Ad-CD154 versus A20 cells that were treated with the optimal concentration of ISS-ODN. A20 cells could stimulate syngeneic splenocytes to secrete IFN-gamma and to proliferate in an autologous MLR when they were incubated with ISS-ODN, but not with a control ODN. Furthermore, A20 cells also could function as effective stimulator cells in the autologous MLR when transduced with Ad-CD154, but not with an Ad vector encoding an irrelevant transgene. However, Ad-CD154-infected A20 cells were significantly more effective APC than oligonucleotide-treated cells, inducing greater T cell proliferation and 10-fold higher-level production of IFN-gamma than equivalent numbers of A20 cells that had been treated with ISS-ODN. Also, splenocytes from BALB/c mice vaccinated with Ad-CD154-infected A20 cells secreted higher amounts of IFN-gamma compared to mice vaccinated with ISS-ODN-treated A20 cells, as determined by ELISA and ELISPOT assays. In this context, Ad-CD154-infected A20 cells, but not ISS-ODN-treated A20 cells or A20 cells infected with a control Ad vector, could induce protective immunity against a lethal challenge with A20 cells in BALB/c in adoptive transfer experiments. We conclude that transduction of A20 cells with Ad-CD154 is more effective in inducing protective anti-lymphoma immunity than treatment of A20 cells with ISS-ODN.

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Refine Search

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(L8).PGPB.	92

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Search: L10

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Search History

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Set Name Side by Side	Query	Hit Count	Set Name Result Set	Set Name Grid
Prior Art Searches				
	DB=PGPB; PLUR=YES; OP=ADJ			
L10	L8	92	L10	L10
	DB=USPT; PLUR=YES; OP=ADJ			
L9	L8	49	L9	L9
	DB=PGPB,USPT; PLUR=YES; OP=ADJ			
L8	L7 same (secretable or secreted or secretion)	141	L8	L8
L7	(adenoviral or adenovirus)same(vector) same(cytokine$) same(secret$)	187	L7	L7
	DB=PGPB,USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			

L6	(adenoviral or adenovirus)same(vector) same(cd40L or cd40 adj ligand or cd154) and (cd40L or cd40 adj ligand or cd154) same(secret$)	47	L6	L6
L5	(adenoviral or adenovirus)same(vector) same(cd40L or cd40 adj ligand or cd154)same(secret$)	33	L5	L5
L4	(adenoviral or adenovirus)same(vector) and (cd40L or cd40 adj ligand or cd154)same(secret$)	606	L4	L4
L3	(l1 or L2) and (adenoviral or adenovirus)same(vector) and (cd40L or cd40 adj ligand or cd154)same(secret$)	14	L3	L3
L2	zhang.in.	276045	L2	L2
L1	deisseroth.in.	62	L1	L1

END OF SEARCH HISTORY

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8161–8166, July 1999
Medical Sciences

Targeting tumor vasculature endothelial cells and tumor cells for immunotherapy of human melanoma in a mouse xenograft model

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Contributed by Alan Garen, May 18, 1999

ABSTRACT **An immunotherapy treatment for cancer that targets both the tumor vasculature and tumor cells has shown promising results in a severe combined immunodeficient mouse xenograft model of human melanoma. The treatment involves systemic delivery of an immunoconjugate molecule composed of a tumor-targeting domain conjugated to the Fc effector domain of human IgG1. The effector domain induces a cytolytic immune response against the targeted cells by natural killer cells and complement. Two types of targeting domains were used. One targeting domain is a human single-chain Fv molecule that binds to a chondroitin sulfate proteoglycan expressed on the surface of most human melanoma cells. Another targeting domain is factor VII (fVII), a zymogen that binds with high specificity and affinity to the transmembrane receptor tissue factor (TF) to initiate the blood coagulation cascade. TF is expressed by endothelial cells lining the tumor vasculature but not the normal vasculature, and also by many types of tumor cells including melanoma. Because the binding of a fVII immunoconjugate to TF might cause disseminated intravascular coagulation, the active site of fVII was mutated to inhibit coagulation without affecting the affinity for TF. The immunoconjugates were encoded as secreted molecules in a replication-defective adenovirus vector, which was injected into the tail vein of severe combined immunodeficient mice. The results demonstrate that a mutated fVII immunoconjugate, administered separately or together with a single-chain Fv immunoconjugate that binds to the tumor cells, can inhibit the growth or cause regression of an established human tumor xenograft. This procedure could be effective in treating a broad spectrum of human solid tumors that express TF on vascular endothelial cells and tumor cells.**

An earlier study showed that immunoconjugates composed of an anti-human melanoma single-chain Fv (scFv) targeting domain, conjugated to the Fc region of human IgG1 as the effector domain, mediated specific lysis *in vitro* of human melanoma cells by natural killer cells and complement (1). The scFv molecules were isolated from a fusion-phage display library derived from the antibody repertoire of a melanoma patient who was vaccinated with autologous tumor cells (2, 3). The cognate antigen for the immunoconjugates is the melanoma-associated chondroitin sulfate proteoglycan MCSP, which is expressed predominately on the surface of most melanoma cells (1, 4). The study reported here was designed to test further the therapeutic potential of an anti-MCSP scFv immunoconjugate in a severe combined immunodeficient (SCID) mouse xenograft model of human melanoma.

Also included in this study is another type of anti-tumor immunoconjugate containing as the targeting domain the zymogen factor VII (fVII), which binds with high affinity and specificity to the transmembrane receptor tissue factor (TF), and after activation initiates blood coagulation (5). TF is expressed by endothelial cells lining the vasculature of solid tumors but not of normal tissues (6, 7) and also is expressed by many types of tumor cells (8). Thus, TF provides a target on both the tumor vasculature and tumor cells for a fVII immunoconjugate. Binding of a fVII immunoconjugate to tumor vasculature endothelial cells should result in lysis of the endothelial cells and the loss of vascular functions essential for tumor growth and survival (9). In a human melanoma xenograft growing in SCID mice, the TF targets include human TF expressed by the tumor cells and mouse TF expressed by the endothelial cells in the tumor vasculature. Because mouse fVII (mfVII) binds strongly both to human TF and mouse TF, unlike human fVII that binds strongly to human TF but weakly to mouse TF (10), mfVII was chosen as the targeting domain for the fVII immunoconjugate. The complex formed between TF and fVII can result in disseminated intravascular coagulation (DIC), a potentially lethal complication associated with cancer (11). To prevent the possible occurrence of DIC in mice treated systemically with a fVII immunoconjugate, the active site of the targeting domain was mutated to inhibit initiation of the coagulation pathway without affecting the affinity for TF (12).

These two types of immunoconjugates, containing either an anti-MCSP scFv (*G71–1*) (3) or a mfVII active site mutant (*mfVIIasm*) as the tumor-targeting domain conjugated to the Fc region of human IgG1, were separately encoded in a replication-defective adenoviral vector (13), and the adenovirus was injected into the tail vein of SCID mice carrying a human melanoma xenograft. The cells infected by the adenovirus synthesized and secreted the encoded immunoconjugate into the blood for at least 1 week. The secreted immunoconjugates should be transported in the blood to the vasculature of the xenograft, where the *mfVIIasm* immunoconjugate can interact with the TF targets on the tumor vascular endothelial cells. Because the walls of the tumor vasculature are leaky (14), the immunoconjugates also should interact with the MCSP and TF targets on the melanoma cells. The Fc domain of the immunoconjugates should activate an immune response against the targeted tumor vascular endothelial cells and tumor cells by components of the immune system that remain functional in SCID mice, such as natural killer cells and complement. The results reported here demonstrate that the growth of an established human melanoma xenograph, expressing a low or high level of TF, can be inhibited by i.v. injections into the SCID mice of the adenoviral vectors encoding these immunoconjugates.

MATERIALS AND METHODS

Cell Lines. The melanoma cell lines LXSN, TF2, and LXSN/VEGF were derived from the human melanoma line

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Abbreviations: scFv, single-chain Fv; MCSP, melanoma-associated chondroitin sulfate proteoglycan; SCID, severe combined immunodeficient; fvII, factor VII; mfVII, mouse fVII; *mfVIIasm*, mfVII active site mutant; TF, tissue factor; VEGF, vascular endothelial growth factor; CHO, Chinese hamster ovary.

*To whom reprint requests should be addressed.

YU-SIT1 by retroviral-mediated transfection and cloning (15). The LXSN line was transfected with the control retrovirus and expresses a low level of TF. The TF2 line was transfected with a retrovirus encoding TF cDNA and expresses a high level of TF. The LXSN/VEGF line was transfected with a retrovirus encoding vascular endothelial growth factor (VEGF) cDNA and expresses high level of VEGF. The human kidney line 293 was purchased from the American Type Culture Collection.

Plasmid Vector. The construction of the plasmid vector encoding the scFv (*G7I–1*) immunoconjugate has been described (1). For the construction of the vector encoding the mfVII immunoconjugate, the mfVII cDNA was amplified by PCR from a mouse liver cDNA library (Quick-Clone cDNA, CLONTECH) by using the 5′ primer ACGATCTTAAGCTTCCCCACAGTCTCATCATGGTTCCA and the 3′ primer ACGGTAACGGATCCCAGTAGTGGGAGTCGGAAAACCCCC (16). The amplified mfVII cDNA, which contains the leader and coding sequences without a stop codon, was cloned into the *Hin*dIII and *Bam*HI sites of the pcDNA3.1(+) vector (Invitrogen) in-frame with a cDNA encoding the human IgG1 Fc domain (1). The vector DNA was amplified in HB101 competent cells (Life Technologies, Grand Island, NY) and sequenced. The active site of mfVII cDNA was mutated by substituting an alanine codon for Lys-341 (12). The mutagenesis procedure was done as described in the QuickChange site-directed mutagenesis manual (Stratagene). The 5′ primer was GGTACCAAGGACGCCTGCGCGGGTGACAGCGGTGGCCCA, and the 3′ primer was TGGGCCACCGCTGTCACCCGCGCAGGCGTCCTTGGTACC. The mfVII cDNA with the active site mutation is designated *mfVIIasm*. The plasmid containing *mfVIIasm* cDNA was transformed into HB101 competent cells, and transformed colonies were selected on 2×TY/carbenicillin agar. The sequence of the plasmid DNA showed a substitution of an alanine codon (GCG) for the Lys-341 codon (AAG) in the *mfVIIasm* DNA.

Synthesis of Immunoconjugates in Chinese Hamster Ovary (CHO) Cells. The procedures for transfecting the immunoconjugate cDNAs into CHO cells and isolating clones were described (1). The transfected CHO cells were cultured in CHO serum-free medium (EX-CELL 301, JRH Biosciences, Lenexa, KS); for synthesis of the *mfVIIasm* immunoconjugate, the CHO serum-free medium was supplemented with vitamin K1 (Sigma) to a final concentration of 1 μg/ml (17). The immunoconjugates were purified by affinity chromatography on Protein A beads (Pierce) and were concentrated and desalted by centrifugation through an Ultrafree-15 Biomax-50 filter (Millipore) and adjusted to 10 mM Tris·HCl, pH 8.0. The immunoconjugate concentrations were measured by the Bio-Rad protein assay procedure.

Fluorescence-Activated Cell Sorting. Melanoma cells were harvested in nonenzymatic dissociation solution (Sigma), washed and resuspended in TBS/BSA/Ca^{2+} (10 mM Tris·HCl, pH 7.4/150 mM NaCl/20 mM $CaCl_2$/1% BSA/0.1% NaN_3). An immunoconjugate was added (5 μg/ml final concentration), and the cells were incubated for 30 min either at 37°C for the *mfVIIasm* immunoconjugate or on ice for the *G7I–1* immunoconjugate; the control cells were incubated without added immunoconjugate. After incubation the cells were washed with TBS/BSA, incubated 30 min on ice with fluorescein-labeled anti-human Fc γ-chain (Vector Laboratories), and analyzed on a Becton Dickinson FACsort instrument.

Adenoviral Vectors. The adenoviral vector system consists of the shuttle vectors pAdTrack-CMV and pShuttle-CMV and the backbone vector pAdEasy-1 (13). The immunoconjugate cDNAs were isolated from the pcDNA3.1 plasmid vectors by digestion with *Hin*dIII followed by Klenow fragment to fill in the 3′ recessed end, and then they were digested with *Not*I to release the cDNA insert, which was purified by agarose gel electrophoresis. The shuttle vectors first were digested with *Kpn*I followed by Klenow fragment, and then were digested with *Not*I. The immunoconjugate cDNAs were ligated into the shuttle vectors by incubation with T4 DNA ligase at 16°C overnight, and the shuttle vectors were transformed into HB101 competent cells by heat shock. Transformed colonies were selected on 2×TY/kanamycin agar, and the shuttle vectors were extracted and purified. The purified shuttle vectors and pAdTrack-CMV DNAs were digested with *Pme*I at 37°C for 2 hr. A mixture of 500 ng shuttle vector DNA and 100 ng pAdEasy-1 DNA was electroporated into BJ5183 competent cells, and the cells were shaken at 37°C for 15 min and plated on LB/kanamycin agar. The plates were incubated at 37°C overnight, and transformed colonies were isolated. The plasmid DNAs were purified from minipreps and screened for recombinant adenoviral DNA by electrophoresis on 0.6% agarose gels.

The recombinant adenoviral DNAs encoding the immunoconjugates were transfected into 1×10^5 293 cells, following the protocol described above for transfecting CHO cells. The cells were collected 7 days after transfection, and the adenoviruses were released by three freeze-thaw cycles and amplified by infecting 293 cells in one 150-mm culture plate. After 2 days the adenoviruses were harvested as described above and amplified again by infecting 293 cells in 20 culture plates. The amplified adenoviruses were harvested 2 days later and purified by centrifugation in CsCl. The final yields usually were about 10^{13} virus particles as estimated from the absorbance at 260 nm; the conversion is 1 OD unit = 1×10^{12} particles. The purified adenoviruses were dialyzed against PBS and stored at −80°C.

SCID Mice Experiments. All animal protocols were approved by the Yale Institutional Committee. The SCID mice were 4- to 5-week-old females from Taconic Farms. The mice were injected s.c. into the right rear flank with 5×10^5 TF2 or LXSN human melanoma cells. After the tumors had grown to a palpable size below the skin surface (≈5 mm^3) or to a larger size above the skin surface (≈50 mm^3), the mice were injected via the tail vein with the adenoviral vector encoding an immunoconjugate, or as a control with the adenoviral vector that does not encode an immunoconjugate. The concentration of immunoconjugate protein secreted into blood was measured by collecting about 0.1 ml of blood from one eye into a microcapillary tube coated with heparin and centrifuging the blood to remove cells. The supernatant plasma was diluted with sodium bicarbonate buffer, pH 9.6 and distributed into wells of probind assay plates (Falcon), and the plates were incubated first at 37°C for 2 hr and then at 4°C overnight. The wells were blocked with 5% nonfat milk in PBS for 30 min and washed three times with PBS, and a peroxidase-labeled anti-human IgG antibody diluted 1:2,000 in 5% nonfat milk was added to the wells. The plates were incubated for 1 hr at room temperature and washed in PBS, and the peroxidase substrate OPD was added and absorbance was measured at 490 nm in a microplate reader. The protein standard was human IgG (Sigma), which we purified by chromatography on Protein A beads.

The size of a tumor appearing on the skin of a SCID mouse was measured in two dimensions with a caliper, and the tumor volume was estimated by the formula $(\text{width})^2\,(\text{length})/2$. At the end of an experiment, the mice were dissected, and the tumors were weighed. The organs were examined for morphological evidence of damage, and paraffin sections were prepared for histological examination.

Immunohistochemistry. Paraffin sections of the tumors and organs were incubated in PBS + 0.3% H_2O_2 for 30 min and blocked in TBS/BSA buffer for 30 min. A solution containing 10 μg/ml the *mfVIIasm* immunoconjugate in TBS/BSA/Ca^{2+} buffer, or as a control the buffer without the immunoconjugate, was added to the sections and incubated at 37°C for 1 hr. After washing three times in the same buffer, the sections were incubated at room temperature for 1 hr with anti-human

γ-chain antibody labeled with alkaline phosphatase, stained with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium, which produces a blue color, and counterstained with methyl green.

RESULTS

Properties of the Immunoconjugates. The scFv (*G71–1*) and the *mfVIIasm* immunoconjugates were synthesized in CHO cells and purified from the culture medium by affinity chromatography on Protein A beads. An earlier analysis by SDS/PAGE showed that the *G71–1* immunoconjugate is composed of two identical chains, presumably coupled by disulfide bridges between the hinge regions of the Fc domains (1). The same result was obtained with the *mfVIIasm* immunoconjugate (data not shown). Because the *mfVIIasm* immunoconjugate has two targeting domains, as compared with the single targeting domain in the monomeric endogenous fVII molecule, it can bind cooperatively to two TF molecules, resulting in stronger binding than endogenous fVII to cells expressing TF. A competitive fluorescence-activated cell sorting assay (Fig. 1) showed that human fVIIa competes on an equimolar basis with the *mfVIIasm* immunoconjugate for binding to half of the accessible sites on human melanoma cells, probably because only one of the targeting domains on the immunoconjugate molecule can bind to TF at these sites. The binding of the *mfVIIasm* immunoconjugate to the remaining sites could not be competed in the presence of a 10-fold excess of human fVIIa, suggesting that both targeting domains of the immunoconjugate molecule can bind at these sites and provide a strong avidity effect. It appears that only about half of the TF molecules on the melanoma cells are sufficiently close to a second TF molecule to form a cooperative binding site for both targeting domains on a *mfVIIasm* immunoconjugate.

The xenografts for the immunotherapy tests were generated from the human melanoma lines LXSN and TF2, which express, respectively, low or high levels of TF. The *mfVIIasm* immunoconjugate binds more extensively to the TF2 cells than to the LXSN cells as determined by fluorescence-activated cell sorting (Fig. 2), consistent with the higher level of TF expression by TF2 cells. The *mfVIIasm* immunoconjugate also was tested by immunohistochemistry for binding to sections of a human melanoma xenograft generated from the melanoma line LXSN/VEGF, which produces a high level of VEGF, resulting in a densely vascularized xenograft. Binding occurred to the tumor vascular endothelial cells as well as to the tumor cells (Fig. 3), indicating that TF is expressed by both cell types in the xenograft. Immunohistochemistry tests with sections of normal mouse liver, kidney, lung, and brain showed that the *mfVIIasm* immunoconjugate does not bind to vascular endothelial cells in these tissues, consistent with other evidence that TF is not expressed by vascular endothelial cells of nontumorous tissues (6, 7).

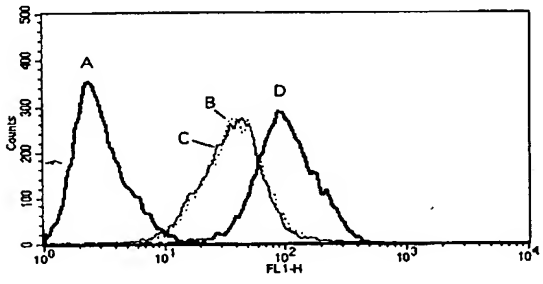

FIG. 1. Competition between human fVIIa and the *mfVIIasm* immunoconjugate for binding to TF2 cells. The assays were done by fluorescence-activated cell sorting. Curve A: Control without fVIIa or *mfVIIasm* immunoconjugate. Curve B: Equimolar mixture of fVIIa and *mfVIIasm* immunoconjugate (25 nM each). Curve C: 10× molar excess of fVIIa to *mfVIIasm* immunoconjugate (250 nM/25 nM). Curve D: *mfVIIasm* immunoconjugate only (25 nM).

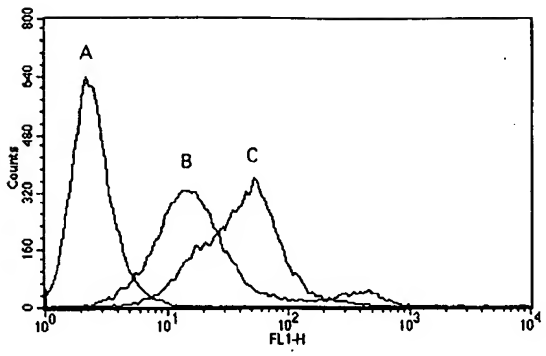

FIG. 2. Fluorescence-activated cell sorting assays for binding of the *mfVIIasm* immunoconjugate to LXSN and TF2 cells. Curve A: TF2 cells without *mfVIIasm*; curve B: LXSN cells with *mfVIIasm*; curve C: TF2 cells with *mfVIIasm*.

Immunotherapy Tests. For systemic delivery to SCID mice, each immunoconjugate was encoded as a secreted molecule in the replication-defective adenoviral vector system based on pAdEasy-1 (13), and the vectors were injected into the tail vein of mice that had first been injected s.c. with human melanoma cells. The initial immunotherapy tests involved injecting each vector separately, and both vectors together, into the mice that had developed a palpable TF2 xenograft. A total of three injections were administered at weekly intervals, and the experiment was terminated 6 days after the last injection. The concentration of the immunoconjugates in the blood was monitored by ELISA after the first and second injections (Fig. 4). The average concentration after the first injection was 4 mg/ml for the *G71–1* immunoconjugate and 0.04 mg/ml for the *mfVIIasm* immunoconjugate, indicating that the rate of synthesis was about 100-fold higher for the *G71–1* immunoconjugate than for the *mfVIIasm* immunoconjugate. The concentration of each immunoconjugate increased after the second injections, indicating that additional cells had been infected by the adenoviruses. The growth of the xenografts was monitored by measuring in two dimensions the size of the tumor appearing on the skin surface, and by using the measurements to estimate the tumor volume (Fig. 5). In the control mice injected with the adenovirus that does not encode an immunoconjugate, the tumor grew continuously at a relatively fast rate, reaching an average volume of about 2,000 mm^3 after 20 days. In the mice injected with an adenovirus encoding an immunoconjugate, tumor growth was inhibited; the inhibition was stronger for the *mfVIIasm* immunoconjugate than for the *G71–1* immunoconjugate. All of the mice remained active and appeared healthy at the end of the experiment, and histological examination of the liver, spleen, lung, kidney, and brain did not show any evidence of necrosis, clotting, or bleeding (data not shown). However, many of the liver cells were enlarged, probably because the adenoviral vectors infect mainly liver cells (18), which continuously synthesize high levels of the encoded immunoconjugates. The tumor weights after autopsy were lower in the mice treated with the immunoconjugates than in the control mice, consistent with the estimated tumor volumes (Fig. 6). The strongest reduction of tumor weight occurred in the mice treated with both immunoconjugates.

The next two experiments were designed to test two parameters that could affect the therapeutic efficacy of the immunoconjugates, namely the initial size of the xenograft and the level of TF expression by the melanoma cells. (*i*) The preceding immunotherapy tests involved palpable melanoma xenografts that had grown to an estimated volume of about 5 mm^3,

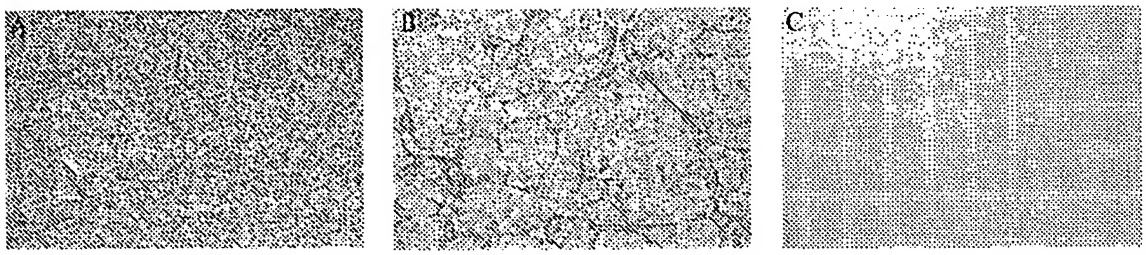

FIG. 3. Immunohistochemical assay for binding of the *mfVIIasm* immunoconjugate to tumor cells and tumor vascular endothelial cells in an LXSN/VEGF xenograft grown in SCID mice. The second antibody was anti-human γ-chain labeled with alkaline phosphatase, and the substrate was 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium, which produces a blue color; the counterstain was methyl green. (*A*) Control stained with hematoxylin + eosin showing extensive vascularization of the xenograft. (*B*) Immunohistochemistry with the *mfVIIasm* immunoconjugate showing intense staining of both the vasculature and tumor cells. (*C*) Immunohistochemical control without the *mfVIIasm* immunoconjugate. Magnification: ×85.

corresponding to a small tumor in humans. To test the therapeutic efficacy of the immunoconjugates against a larger xenograft, TF2 xenografts were allowed to grow to an estimated volume of about 50 mm^3 before starting tail vein injections of the two adenoviral vectors. The mice received four injections during a period of 3 weeks, and the experiment was terminated 2 days after the last injection. The average tumor volume in the mice injected with the adenoviruses encoding the immunoconjugates was about the same at the end as at the start of the experiment, in contrast to the average tumor volume in the mice injected with the control adenovirus, which increased by a factor of about 27 during the same period (Fig. 7). These results show that tumor growth is inhibited as effectively with the larger tumor as with the smaller tumor. One of the five mice injected with the adenovirus encoding the immunoconjugates died 5 days after the third injection; the cause of death could not be determined because the mouse was not recovered in time for examination. (*ii*) A parameter that might affect the efficacy of the *mfVIIasm* immunoconjugate is the level of TF expression, which varies among different tumors (8). To study the effect of varying the expression of TF by the melanoma cells in a xenograft, the melanoma line LXSN was used to generate a xenograft expressing a low level of TF, for comparison with the xenograft generated from the related line TF2, which expresses a higher level of TF (15). After the xenografts reached a palpable size, the mice received during the next 3 weeks five injections of the adenovirus encoding the *fVIIasm* immunoconjugate or the control adenovirus (Fig. 8). In the five mice injected with the control adenovirus the xenograft grew continuously, the average volume increasing to 1,350 mm^3 on the second day after the last injection. During the same period the average volume of the xenografts in the mice injected with the *mfVIIasm* immunoconjugate increased to 20 mm^3, indicating that the inhibition of tumor development is comparable for the *LXSN* and *TF2* xenografts (compare Figs. 5 and 8). The autopsies performed 1 day after the last injection showed that the xenograft had been eradicated in two of the five mice injected with the adenovirus encoding the *mfVIIasm* immunoconjugate; the average tumor weight in the other three mice was 0.11 g as compared with the average weight of 0.75 g in the five mice injected with the control adenovirus. The small tumors recovered from these three mice showed extensive regions of cell necrosis, which did not occur in the larger tumors from the control mice (Fig. 9). All of the mice appeared healthy at the end of this experiment, but a morphological examination of the dissected mice revealed damage to the liver and spleen in the five mice injected with the adenovirus encoding the *mfVIIasm* immunoconjugate. Histological examination of the liver and spleen showed that many of the liver cells were enlarged, and the spleen was extensively infiltrated with erythrocytes. Enlarged liver cells also occurred in a previous experiment after three injections of the adenovirus encoding the *mfVIIasm* immunoconjugate, but the spleen was normal, indicating that the defects in the spleen developed in the course of the last two injections. One of the mice also had a subdural brain hemorrhage, which did not occur in other mice from this experiment or any of the previous experiments. It is uncertain whether this defect was induced by the binding of the *mfVIIasm* immunoconjugate to

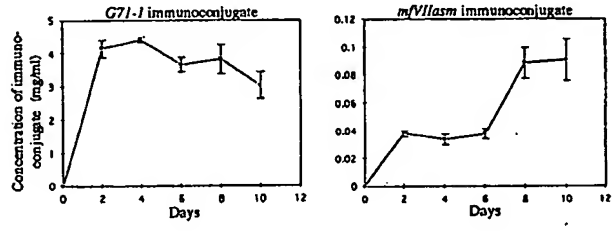

FIG. 4. Concentrations of the *G71–1* and *mfVIIasm* immunoconjugates in the blood of SCID mice after i.v. injections of the adenovirus encoding each immunoconjugate. The mice were injected on days 0 and 7 with 2×10^{11} adenovirus encoding the *G71–1* immunoconjugate or with 4×10^{11} adenovirus encoding the *mfVIIasm* immunoconjugate. The concentration of the encoded immunoconjugate in the blood was determined by ELISA. Each point is the average of the concentration for the five mice in each group.

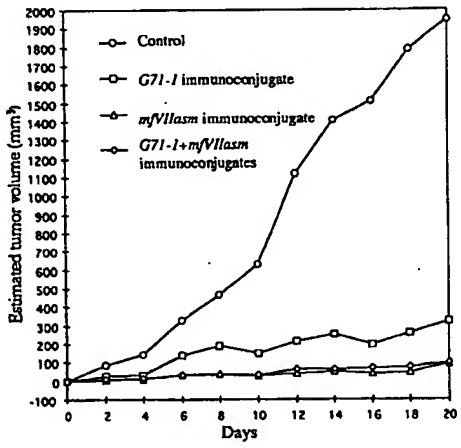

FIG. 5. Inhibitory effect of the *G71–1* and *mfVIIasm* immunoconjugates on the growth of a TF2 xenograft. For each curve five SCID mice were injected s.c. with 5×10^5 TF2 cells. When the xenografts had grown to a palpable size, the mice received tail vein injections on days 0, 7, and 14 of the adenoviruses indicated. The amount of adenovirus injected was 4×10^{11} for the control, 2×10^{11} for the adenovirus encoding the *G71–1* immunoconjugate, and 4×10^{11} for the adenovirus encoding the *mfVIIasm* immunoconjugate. The estimated tumor volumes are the averages for the five mice in each group.

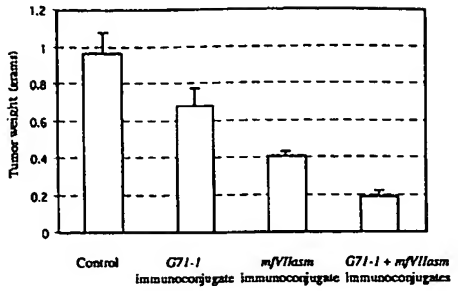

FIG. 6. Tumor weights of the xenografts from the experiment reported in Fig. 5. The xenografts were dissected from the mice on day 20, which was 6 days after the last injection of adenovirus. The bar heights are the average weights for the five mice in each group.

TF expressed in the brain vasculature or occurred spontaneously.

DISCUSSION

A SCID mouse xenograft model of human melanoma was used to test the therapeutic potential of an immunotherapy procedure designed to target both the tumor vasculature endothelial cells and tumor cells for cytolysis by the host immune system. The procedure involved systemic delivery to SCID mice of two immunoconjugates, each composed of a tumor-targeting domain conjugated to the Fc region of a human IgG1 heavy chain, forming a homodimeric molecule similar to a Camelid heavy-chain antibody (19). For one type of immunoconjugate, the tumor-targeting domain was the human scFv molecule *G71–1* that binds to the melanoma antigen MCSP (1, 4) expressed by the melanoma cells in the xenografts. For the other type of immunoconjugate, the tumor-targeting domain was a mfVII molecule that binds specifically and tightly to TF, both to mouse TF expressed by the tumor vasculature endothelial cells and to human TF expressed by the melanoma cells in the xenografts. To decrease the risk of disseminated intravascular

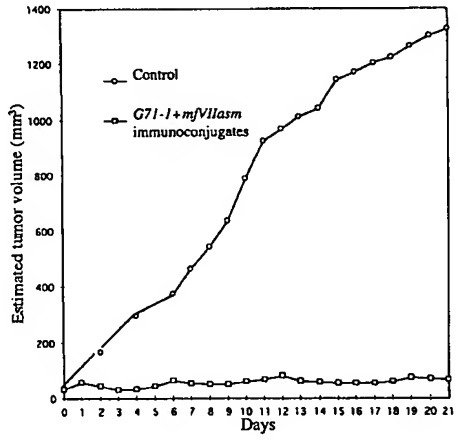

FIG. 7. Inhibitory effect of the *G71–1* and *mfVIIasm* immunoconjugates on the growth of a larger TF2 xenograft. Each mouse was injected s.c. with 5×10^5 TF2 cells, and the xenografts were allowed to grow to an estimated tumor volume of 50 mm^3 on the skin surface (day 1). A mixture of 2×10^{11} adenoviruses encoding the *G71–1* immunoconjugate and 7×10^{11} adenoviruses encoding the *mfVIIasm* immunoconjugate was injected into the tail vein of five mice on days 1, 6, 12, and 19. As a control five mice were injected with 4×10^{11} adenoviruses that did not encode an immunoconjugate. The estimated tumor volumes are the averages for the five mice in each group. One of the mice injected with the adenoviruses encoding the immunoconjugates was found dead on day 17; the estimated tumor volumes on subsequent days are the averages for the remaining four mice.

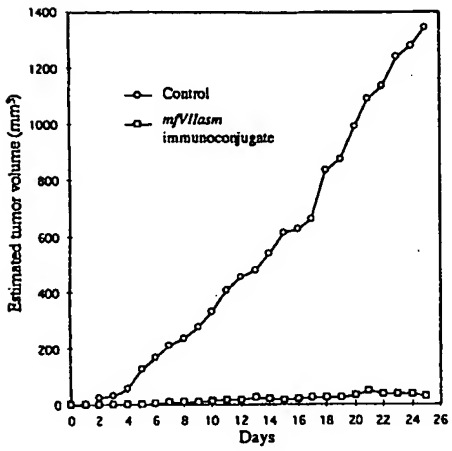

FIG. 8. Inhibitory effect of the *mfVIIasm* immunoconjugate on the growth of an LXSN xenograft. The mice were injected s.c. with 5×10^5 LXSN cells, and when the xenograft had grown to a palpable size (day 0) five mice were injected with 9×10^{11} adenoviruses encoding the *mfVIIasm* immunoconjugate, and five mice were injected with 4×10^{11} control adenoviruses. Additional injections were done on days 7, 13, 21, and 24, and on day 25 the mice were dissected for morphological and histochemical examination. The estimated tumor volumes are the averages for the five mice in each group.

coagulation that might result from the binding of a fVII immunoconjugate to TF, an active site mutation was introduced into the mfVII targeting domain (*mfVIIasm*), inhibiting the proteolytic activity required to initiate the blood coagulation pathway.

An earlier *in vitro* study showed that the *G71–1* immunoconjugate mediates cytolysis of cultured human melanoma cells by natural killer (NK) cells and complement (1). Because SCID mice retain the capacity to produce functional NK cells and complement, the immunoconjugates also could mediate cytolysis of the targeted tumor cells and vascular endothelial cells of a human melanoma xenograft growing in SCID mice. Systemic delivery of the immunoconjugates to SCID mice was achieved by tail vein injections of a replication-defective adenoviral vector encoding the immunoconjugates, which were secreted into the blood for at least 1 week after each injection. The mice first were injected s.c. with a human melanoma cell line that expresses either a low or high level of TF, and the resulting xenograft was allowed to grow into a small (≈5 mm^3) or larger (≈50 mm^3) tumor before starting injections of the adenoviral vectors. Further growth of all the xenografts was prevented for the 3- to 4-week period of the experiments by multiple injections of the adenovirus encoding the *mfVIIasm* immunoconjugate, administered separately or together with the adenovirus encoding the *G71–1* immunoconjugate; in some of the mice the xenograft completely regressed. In the control mice, which were injected with an adenovirus that did not encode an immunoconjugate, the average volume of the xenografts increased by a factor of about 25 during the same period. In the mice receiving five injections of the adenoviral vectors encoding the immunoconjugates, many of the liver cells were enlarged and the spleen was infiltrated with erythrocytes. The defects were not caused by the secreted immunoconjugates, which do not bind to the liver or spleen cells. The primary cause probably is the continuous high-level synthesis of the encoded immunoconjugates by the liver cells, which are the mouse cells predominately infected by i.v. injected adenoviral vectors (18). The enlarged liver cells could have produced an increased blood pressure in the spleen, causing blood vessels to rupture. If these defects also can occur in a clinical setting, the problem might be corrected by changing the dose, schedule, or route of injection for the

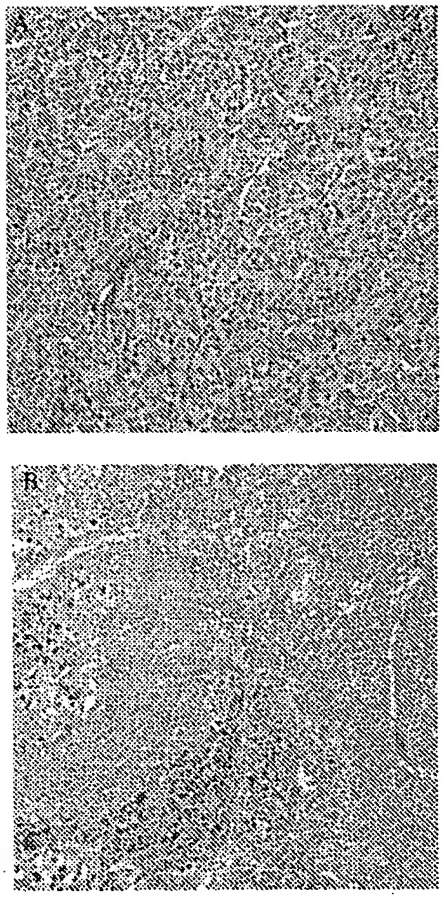

FIG. 9. Histochemistry of the LXSN xenografts from the experiment reported in Fig. 8. The xenografts were dissected on day 25 and embedded in paraffin, and sections were stained with hemotoxylin + eosin. (*A*) Xenograft from a control mouse injected with the adenovirus that does not encode an immunoconjugate. (*B*) Xenograft from a mouse injected with the adenovirus encoding the *mfVIIasm* immunoconjugate. Magnification: ×245.

adenoviral vectors, or by using a different type of vector. Another possible option is to administer the immunoconjugates directly as proteins.

Although the immunoconjugate concentration in the blood of SCID mice injected with an adenoviral vector was about 100-fold higher for the *G71–1* immunoconjugate than for the *mfVIIasm* immunoconjugate, the inhibitory effect on a human melanoma xenograft nevertheless was stronger with the *mfVIIasm* immunoconjugate. A key advantage of the *mfVIIasm* immunoconjugate is the binding that occurs to tumor vascular endothelial cells as well as to tumor cells, in contrast to the *G71–1* immunoconjugate that binds only to melanoma cells. The binding to the tumor vasculature should be tumor specific, because TF is not expressed by the normal vasculature. Although TF is expressed by several other normal tissues, such as brain, lung, and kidney glomeruli, these TF molecules are not accessible to endogenous fVII or a fVII immunoconjugate because the blood vessel walls form a barrier separating larger blood components from adjacent cells. However, tumor blood vessels are leaky (14), allowing access to TF expressed by tumor cells. Thus, a human *fVIIasm* immunoconjugate could be an effective therapeutic agent for a broad spectrum of human tumors expressing TF on the vascular endothelial cells and tumor cells. The therapeutic efficacy of a human *fVIIasm* immunoconjugate could be enhanced by also administering a human scFv immunoconjugate that binds to a tumor target other than TF.

In considering a clinical test of the immunoconjugates, which might require maintaining an adequate titer in the patient's blood for a prolonged period, an immune rejection response to the immunoconjugates and/or the adenoviral vector could be a potential obstacle. Because the tumor-targeting and Fc effector domains of the immunoconjugates are derived from human sources for clinical protocols, the immunoconjugates should be tolerated by the human immune system. Although it was possible to use the adenoviral delivery system for repeated injections in SCID mice, an adenovirus might be too immunogenic in patients for this purpose. To avoid immune rejection of the vector, a nonimmunogenic vector could be substituted for the adenovirus, or the immunoconjugates could be administered directly as proteins.

We are grateful for the contributions of our colleagues, Dr. Michael Bromberg, who generously provided the human melanoma cell lines LXSN, TF2, and LXSN/VEGF, and Dr. Baiyang Wang, who provided advice and assistance for the experiments. The mouse autopsies and examinations were done by Gordon Terwilliger. Dr. Albert Deisseroth, Section Chief of Medical Oncology, Yale University School of Medicine, provided essential resources and facilities for producing adenoviral vectors. This project was supported by U.S. Public Health Service Grant PO1 HL29010 and a generous gift from private donors (S. L. Misrock and A. M. Fox).

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Medical Sciences

Targeting tumor vasculature endothelial cells and tumor cells for immunotherapy of human melanoma in a mouse xenograft model

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Contributed by Alan Garen, May 18, 1999

ABSTRACT **An immunotherapy treatment for cancer that targets both the tumor vasculature and tumor cells has shown promising results in a severe combined immunodeficient mouse xenograft model of human melanoma. The treatment involves systemic delivery of an immunoconjugate molecule composed of a tumor-targeting domain conjugated to the Fc effector domain of human IgG1. The effector domain induces a cytolytic immune response against the targeted cells by natural killer cells and complement. Two types of targeting domains were used. One targeting domain is a human single-chain Fv molecule that binds to a chondroitin sulfate proteoglycan expressed on the surface of most human melanoma cells. Another targeting domain is factor VII (fVII), a zymogen that binds with high specificity and affinity to the transmembrane receptor tissue factor (TF) to initiate the blood coagulation cascade. TF is expressed by endothelial cells lining the tumor vasculature but not the normal vasculature, and also by many types of tumor cells including melanoma. Because the binding of a fVII immunoconjugate to TF might cause disseminated intravascular coagulation, the active site of fVII was mutated to inhibit coagulation without affecting the affinity for TF. The immunoconjugates were encoded as secreted molecules in a replication-defective adenovirus vector, which was injected into the tail vein of severe combined immunodeficient mice. The results demonstrate that a mutated fVII immunoconjugate, administered separately or together with a single-chain Fv immunoconjugate that binds to the tumor cells, can inhibit the growth or cause regression of an established human tumor xenograft. This procedure could be effective in treating a broad spectrum of human solid tumors that express TF on vascular endothelial cells and tumor cells.**

An earlier study showed that immunoconjugates composed of an anti-human melanoma single-chain Fv (scFv) targeting domain, conjugated to the Fc region of human IgG1 as the effector domain, mediated specific lysis *in vitro* of human melanoma cells by natural killer cells and complement (1). The scFv molecules were isolated from a fusion-phage display library derived from the antibody repertoire of a melanoma patient who was vaccinated with autologous tumor cells (2, 3). The cognate antigen for the immunoconjugates is the melanoma-associated chondroitin sulfate proteoglycan MCSP, which is expressed predominately on the surface of most melanoma cells (1, 4). The study reported here was designed to test further the therapeutic potential of an anti-MCSP scFv immunoconjugate in a severe combined immunodeficient (SCID) mouse xenograft model of human melanoma.

Also included in this study is another type of anti-tumor immunoconjugate containing as the targeting domain the zymogen factor VII (fVII), which binds with high affinity and specificity to the transmembrane receptor tissue factor (TF), and after activation initiates blood coagulation (5). TF is expressed by endothelial cells lining the vasculature of solid tumors but not of normal tissues (6, 7) and also is expressed by many types of tumor cells (8). Thus, TF provides a target on both the tumor vasculature and tumor cells for a fVII immunoconjugate. Binding of a fVII immunoconjugate to tumor vasculature endothelial cells should result in lysis of the endothelial cells and the loss of vascular functions essential for tumor growth and survival (9). In a human melanoma xenograft growing in SCID mice, the TF targets include human TF expressed by the tumor cells and mouse TF expressed by the endothelial cells in the tumor vasculature. Because mouse fVII (mfVII) binds strongly both to human TF and mouse TF, unlike human fVII that binds strongly to human TF but weakly to mouse TF (10), mfVII was chosen as the targeting domain for the fVII immunoconjugate. The complex formed between TF and fVII can result in disseminated intravascular coagulation (DIC), a potentially lethal complication associated with cancer (11). To prevent the possible occurrence of DIC in mice treated systemically with a fVII immunoconjugate, the active site of the targeting domain was mutated to inhibit initiation of the coagulation pathway without affecting the affinity for TF (12).

These two types of immunoconjugates, containing either an anti-MCSP scFv (*G71–1*) (3) or a mfVII active site mutant (*mfVIIasm*) as the tumor-targeting domain conjugated to the Fc region of human IgG1, were separately encoded in a replication-defective adenoviral vector (13), and the adenovirus was injected into the tail vein of SCID mice carrying a human melanoma xenograft. The cells infected by the adenovirus synthesized and secreted the encoded immunoconjugate into the blood for at least 1 week. The secreted immunoconjugates should be transported in the blood to the vasculature of the xenograft, where the *mfVIIasm* immunoconjugate can interact with the TF targets on the tumor vascular endothelial cells. Because the walls of the tumor vasculature are leaky (14), the immunoconjugates also should interact with the MCSP and TF targets on the melanoma cells. The Fc domain of the immunoconjugates should activate an immune response against the targeted tumor vascular endothelial cells and tumor cells by components of the immune system that remain functional in SCID mice, such as natural killer cells and complement. The results reported here demonstrate that the growth of an established human melanoma xenograph, expressing a low or high level of TF, can be inhibited by i.v. injections into the SCID mice of the adenoviral vectors encoding these immunoconjugates.

MATERIALS AND METHODS

Cell Lines. The melanoma cell lines LXSN, TF2, and LXSN/VEGF were derived from the human melanoma line

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Abbreviations: scFv, single-chain Fv; MCSP, melanoma-associated chondroitin sulfate proteoglycan; SCID, severe combined immunodeficient; fVII, factor VII; mfVII, mouse fVII; *mfVIIasm*, mfVII active site mutant; TF, tissue factor; VEGF, vascular endothelial growth factor; CHO, Chinese hamster ovary.

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YU-SIT1 by retroviral-mediated transfection and cloning (15). The LXSN line was transfected with the control retrovirus and expresses a low level of TF. The TF2 line was transfected with a retrovirus encoding TF cDNA and expresses a high level of TF. The LXSN/VEGF line was transfected with a retrovirus encoding vascular endothelial growth factor (VEGF) cDNA and expresses high level of VEGF. The human kidney line 293 was purchased from the American Type Culture Collection.

Plasmid Vector. The construction of the plasmid vector encoding the scFv (*G7l–1*) immunoconjugate has been described (1). For the construction of the vector encoding the mfVII immunoconjugate, the mfVII cDNA was amplified by PCR from a mouse liver cDNA library (Quick-Clone cDNA, CLONTECH) by using the 5′ primer ACGATCTTAAGCTTCCCCACAGTCTCATCATGGTTCCA and the 3′ primer ACGGTAACGGATCCCAGTAGTGGGAGTCGGAAAACCCC (16). The amplified mfVII cDNA, which contains the leader and coding sequences without a stop codon, was cloned into the *Hin*dIII and *Bam*HI sites of the pcDNA3.1(+) vector (Invitrogen) in-frame with a cDNA encoding the human IgG1 Fc domain (1). The vector DNA was amplified in HB101 competent cells (Life Technologies, Grand Island, NY) and sequenced. The active site of mfVII cDNA was mutated by substituting an alanine codon for Lys-341 (12). The mutagenesis procedure was done as described in the QuickChange site-directed mutagenesis manual (Stratagene). The 5′ primer was GGTACCAAGGACGCCTGCGCGGGTGACAGCGGTGGCCCA, and the 3′ primer was TGGGCCACCGCTGTCACCCGCGCAGGCGTCCTTGGTACC. The mfVII cDNA with the active site mutation is designated *mfVIIasm*. The plasmid containing *mfVIIasm* cDNA was transformed into HB101 competent cells, and transformed colonies were selected on 2×TY/carbenicillin agar. The sequence of the plasmid DNA showed a substitution of an alanine codon (GCG) for the Lys-341 codon (AAG) in the *mfVIIasm* DNA.

Synthesis of Immunoconjugates in Chinese Hamster Ovary (CHO) Cells. The procedures for transfecting the immunoconjugate cDNAs into CHO cells and isolating clones were described (1). The transfected CHO cells were cultured in CHO serum-free medium (EX-CELL 301, JRH Biosciences, Lenexa, KS); for synthesis of the *mfVIIasm* immunoconjugate, the CHO serum-free medium was supplemented with vitamin K1 (Sigma) to a final concentration of 1 μg/ml (17). The immunoconjugates were purified by affinity chromatography on Protein A beads (Pierce) and were concentrated and desalted by centrifugation through an Ultrafree-15 Biomax-50 filter (Millipore) and adjusted to 10 mM Tris·HCl, pH 8.0. The immunoconjugate concentrations were measured by the Bio-Rad protein assay procedure.

Fluorescence-Activated Cell Sorting. Melanoma cells were harvested in nonenzymatic dissociation solution (Sigma), washed and resuspended in TBS/BSA/Ca^{2+} (10 mM Tris·HCl, pH 7.4/150 mM NaCl/20 mM $CaCl_2$/1% BSA/0.1% NaN_3). An immunoconjugate was added (5 μg/ml final concentration), and the cells were incubated for 30 min either at 37°C for the *mfVIIasm* immunoconjugate or on ice for the *G7l–1* immunoconjugate; the control cells were incubated without added immunoconjugate. After incubation the cells were washed with TBS/BSA, incubated 30 min on ice with fluorescein-labeled anti-human Fc γ-chain (Vector Laboratories), and analyzed on a Becton Dickinson FACsort instrument.

Adenoviral Vectors. The adenoviral vector system consists of the shuttle vectors pAdTrack-CMV and pShuttle-CMV and the backbone vector pAdEasy-1 (13). The immunoconjugate cDNAs were isolated from the pcDNA3.1 plasmid vectors by digestion with *Hin*dIII followed by Klenow fragment to fill in the 3′ recessed end, and then they were digested with *Not*I to release the cDNA insert, which was purified by agarose gel electrophoresis. The shuttle vectors first were digested with *Kpn*I followed by Klenow fragment, and then were digested with *Not*I. The immunoconjugate cDNAs were ligated into the shuttle vectors by incubation with T4 DNA ligase at 16°C overnight, and the shuttle vectors were transformed into HB101 competent cells by heat shock. Transformed colonies were selected on 2×TY/kanamycin agar, and the shuttle vectors were extracted and purified. The purified shuttle vectors and pAdTrack-CMV DNAs were digested with *Pme*I at 37°C for 2 hr. A mixture of 500 ng shuttle vector DNA and 100 ng pAdEasy-1 DNA was electroporated into BJ5183 competent cells, and the cells were shaken at 37°C for 15 min and plated on LB/kanamycin agar. The plates were incubated at 37°C overnight, and transformed colonies were isolated. The plasmid DNAs were purified from minipreps and screened for recombinant adenoviral DNA by electrophoresis on 0.6% agarose gels.

The recombinant adenoviral DNAs encoding the immunoconjugates were transfected into 1×10^5 293 cells, following the protocol described above for transfecting CHO cells. The cells were collected 7 days after transfection, and the adenoviruses were released by three freeze-thaw cycles and amplified by infecting 293 cells in one 150-mm culture plate. After 2 days the adenoviruses were harvested as described above and amplified again by infecting 293 cells in 20 culture plates. The amplified adenoviruses were harvested 2 days later and purified by centrifugation in CsCl. The final yields usually were about 10^{13} virus particles as estimated from the absorbance at 260 nm; the conversion is 1 OD unit = 1×10^{12} particles. The purified adenoviruses were dialyzed against PBS and stored at −80°C.

SCID Mice Experiments. All animal protocols were approved by the Yale Institutional Committee. The SCID mice were 4- to 5-week-old females from Taconic Farms. The mice were injected s.c. into the right rear flank with 5×10^5 TF2 or LXSN human melanoma cells. After the tumors had grown to a palpable size below the skin surface (≈5 mm^3) or to a larger size above the skin surface (≈50 mm^3), the mice were injected via the tail vein with the adenoviral vector encoding an immunoconjugate, or as a control with the adenoviral vector that does not encode an immunoconjugate. The concentration of immunoconjugate protein secreted into blood was measured by collecting about 0.1 ml of blood from one eye into a microcapillary tube coated with heparin and centrifuging the blood to remove cells. The supernatant plasma was diluted with sodium bicarbonate buffer, pH 9.6 and distributed into wells of probind assay plates (Falcon), and the plates were incubated first at 37°C for 2 hr and then at 4°C overnight. The wells were blocked with 5% nonfat milk in PBS for 30 min and washed three times with PBS, and a peroxidase-labeled anti-human IgG antibody diluted 1:2,000 in 5% nonfat milk was added to the wells. The plates were incubated for 1 hr at room temperature and washed in PBS, and the peroxidase substrate OPD was added and absorbance was measured at 490 nm in a microplate reader. The protein standard was human IgG (Sigma), which we purified by chromatography on Protein A beads.

The size of a tumor appearing on the skin of a SCID mouse was measured in two dimensions with a caliper, and the tumor volume was estimated by the formula $(\text{width})^2\,(\text{length})/2$. At the end of an experiment, the mice were dissected, and the tumors were weighed. The organs were examined for morphological evidence of damage, and paraffin sections were prepared for histological examination.

Immunohistochemistry. Paraffin sections of the tumors and organs were incubated in PBS + 0.3% H_2O_2 for 30 min and blocked in TBS/BSA buffer for 30 min. A solution containing 10 μg/ml the *mfVIIasm* immunoconjugate in TBS/BSA/Ca^{2+} buffer, or as a control the buffer without the immunoconjugate, was added to the sections and incubated at 37°C for 1 hr. After washing three times in the same buffer, the sections were incubated at room temperature for 1 hr with anti-human

γ-chain antibody labeled with alkaline phosphatase, stained with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium, which produces a blue color, and counterstained with methyl green.

RESULTS

Properties of the Immunoconjugates. The scFv (*G71–1*) and the *mfVIIasm* immunoconjugates were synthesized in CHO cells and purified from the culture medium by affinity chromatography on Protein A beads. An earlier analysis by SDS/PAGE showed that the *G71–1* immunoconjugate is composed of two identical chains, presumably coupled by disulfide bridges between the hinge regions of the Fc domains (1). The same result was obtained with the *mfVIIasm* immunoconjugate (data not shown). Because the *mfVIIasm* immunoconjugate has two targeting domains, as compared with the single targeting domain in the monomeric endogenous fVII molecule, it can bind cooperatively to two TF molecules, resulting in stronger binding than endogenous fVII to cells expressing TF. A competitive fluorescence-activated cell sorting assay (Fig. 1) showed that human fVIIa competes on an equimolar basis with the *mfVIIasm* immunoconjugate for binding to half of the accessible sites on human melanoma cells, probably because only one of the targeting domains on the immunoconjugate molecule can bind to TF at these sites. The binding of the *mfVIIasm* immunoconjugate to the remaining sites could not be competed in the presence of a 10-fold excess of human fVIIa, suggesting that both targeting domains of the immunoconjugate molecule can bind at these sites and provide a strong avidity effect. It appears that only about half of the TF molecules on the melanoma cells are sufficiently close to a second TF molecule to form a cooperative binding site for both targeting domains on a *mfVIIasm* immunoconjugate.

The xenografts for the immunotherapy tests were generated from the human melanoma lines LXSN and TF2, which express, respectively, low or high levels of TF. The *mfVIIasm* immunoconjugate binds more extensively to the TF2 cells than to the LXSN cells as determined by fluorescence-activated cell sorting (Fig. 2), consistent with the higher level of TF expression by TF2 cells. The *mfVIIasm* immunoconjugate also was tested by immunohistochemistry for binding to sections of a human melanoma xenograft generated from the melanoma line LXSN/VEGF, which produces a high level of VEGF, resulting in a densely vascularized xenograft. Binding occurred to the tumor vascular endothelial cells as well as to the tumor cells (Fig. 3), indicating that TF is expressed by both cell types in the xenograft. Immunohistochemistry tests with sections of normal mouse liver, kidney, lung, and brain showed that the *mfVIIasm* immunoconjugate does not bind to vascular endothelial cells in these tissues, consistent with other evidence that TF is not expressed by vascular endothelial cells of nontumorous tissues (6, 7).

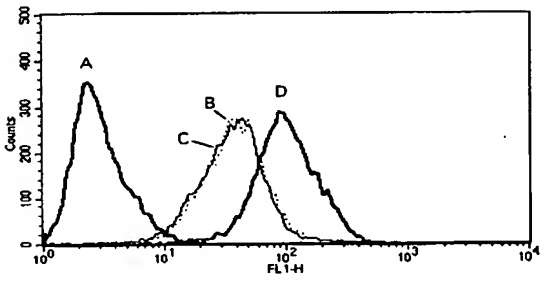

FIG. 1. Competition between human fVIIa and the *mfVIIasm* immunoconjugate for binding to TF2 cells. The assays were done by fluorescence-activated cell sorting. Curve A: Control without fVIIa or *mfVIIasm* immunoconjugate. Curve B: Equimolar mixture of fVIIa and *mfVIIasm* immunoconjugate (25 nM each). Curve C: 10× molar excess of fVIIa to *mfVIIasm* immunoconjugate (250 nM/25 nM). Curve D: *mfVIIasm* immunoconjugate only (25 nM).

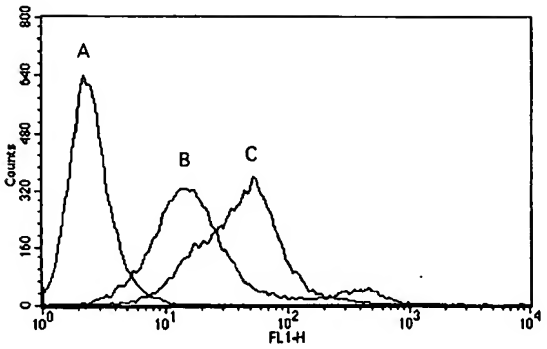

FIG. 2. Fluorescence-activated cell sorting assays for binding of the *mfVIIasm* immunoconjugate to LXSN and TF2 cells. Curve A: TF2 cells without *mfvIIasm*; curve B: LXSN cells with *mfvIIasm*; curve C: TF2 cells with *mfvIIasm*.

Immunotherapy Tests. For systemic delivery to SCID mice, each immunoconjugate was encoded as a secreted molecule in the replication-defective adenoviral vector system based on pAdEasy-1 (13), and the vectors were injected into the tail vein of mice that had first been injected s.c. with human melanoma cells. The initial immunotherapy tests involved injecting each vector separately, and both vectors together, into the mice that had developed a palpable TF2 xenograft. A total of three injections were administered at weekly intervals, and the experiment was terminated 6 days after the last injection. The concentration of the immunoconjugates in the blood was monitored by ELISA after the first and second injections (Fig. 4). The average concentration after the first injection was 4 mg/ml for the *G71–1* immunoconjugate and 0.04 mg/ml for the *mfVIIasm* immunoconjugate, indicating that the rate of synthesis was about 100-fold higher for the *G71–1* immunoconjugate than for the *mfVIIasm* immunoconjugate. The concentration of each immunoconjugate increased after the second injections, indicating that additional cells had been infected by the adenoviruses. The growth of the xenografts was monitored by measuring in two dimensions the size of the tumor appearing on the skin surface, and by using the measurements to estimate the tumor volume (Fig. 5). In the control mice injected with the adenovirus that does not encode an immunoconjugate, the tumor grew continuously at a relatively fast rate, reaching an average volume of about 2,000 mm^3 after 20 days. In the mice injected with an adenovirus encoding an immunoconjugate, tumor growth was inhibited; the inhibition was stronger for the *mfVIIasm* immunoconjugate than for the *G71–1* immunoconjugate. All of the mice remained active and appeared healthy at the end of the experiment, and histological examination of the liver, spleen, lung, kidney, and brain did not show any evidence of necrosis, clotting, or bleeding (data not shown). However, many of the liver cells were enlarged, probably because the adenoviral vectors infect mainly liver cells (18), which continuously synthesize high levels of the encoded immunoconjugates. The tumor weights after autopsy were lower in the mice treated with the immunoconjugates than in the control mice, consistent with the estimated tumor volumes (Fig. 6). The strongest reduction of tumor weight occurred in the mice treated with both immunoconjugates.

The next two experiments were designed to test two parameters that could affect the therapeutic efficacy of the immunoconjugates, namely the initial size of the xenograft and the level of TF expression by the melanoma cells. (*i*) The preceding immunotherapy tests involved palpable melanoma xenografts that had grown to an estimated volume of about 5 mm^3,

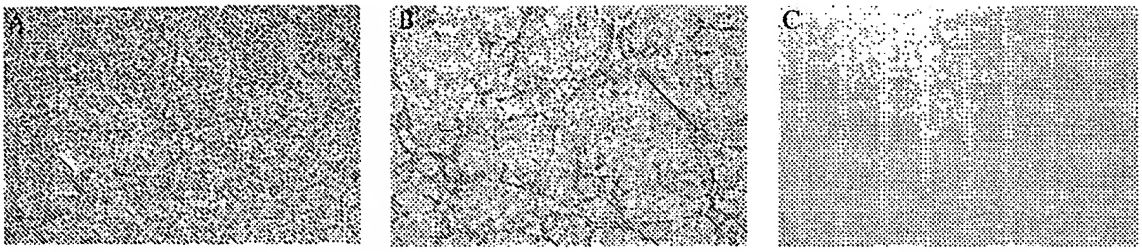

FIG. 3. Immunohistochemical assay for binding of the *mfVIIasm* immunoconjugate to tumor cells and tumor vascular endothelial cells in an LXSN/VEGF xenograft grown in SCID mice. The second antibody was anti-human γ-chain labeled with alkaline phosphatase, and the substrate was 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium, which produces a blue color; the counterstain was methyl green. (*A*) Control stained with hematoxylin + eosin showing extensive vascularization of the xenograft. (*B*) Immunohistochemistry with the *mfVIIasm* immunoconjugate showing intense staining of both the vasculature and tumor cells. (*C*) Immunohistochemical control without the *mfVIIasm* immunoconjugate. Magnification: ×85.

corresponding to a small tumor in humans. To test the therapeutic efficacy of the immunoconjugates against a larger xenograft, TF2 xenografts were allowed to grow to an estimated volume of about 50 mm^3 before starting tail vein injections of the two adenoviral vectors. The mice received four injections during a period of 3 weeks, and the experiment was terminated 2 days after the last injection. The average tumor volume in the mice injected with the adenoviruses encoding the immunoconjugates was about the same at the end as at the start of the experiment, in contrast to the average tumor volume in the mice injected with the control adenovirus, which increased by a factor of about 27 during the same period (Fig. 7). These results show that tumor growth is inhibited as effectively with the larger tumor as with the smaller tumor. One of the five mice injected with the adenovirus encoding the immunoconjugates died 5 days after the third injection; the cause of death could not be determined because the mouse was not recovered in time for examination. (*ii*) A parameter that might affect the efficacy of the *mfVIIasm* immunoconjugate is the level of TF expression, which varies among different tumors (8). To study the effect of varying the expression of TF by the melanoma cells in a xenograft, the melanoma line LXSN was used to generate a xenograft expressing a low level of TF, for comparison with the xenograft generated from the related line TF2, which expresses a higher level of TF (15). After the xenografts reached a palpable size, the mice received during the next 3 weeks five injections of the adenovirus encoding the *fVIIasm* immunoconjugate or the control adenovirus (Fig. 8). In the five mice injected with the control adenovirus the xenograft grew continuously, the average volume increasing to 1,350 mm^3 on the second day after the last injection. During the same period the average volume of the xenografts in the mice injected with the *mfVIIasm* immunoconjugate increased to 20 mm^3, indicating that the inhibition of tumor development is comparable for the *LXSN* and *TF2* xenografts (compare Figs. 5 and 8). The autopsies performed 1 day after the last injection showed that the xenograft had been eradicated in two of the five mice injected with the adenovirus encoding the *mfVIIasm* immunoconjugate; the average tumor weight in the other three mice was 0.11 g as compared with the average weight of 0.75 g in the five mice injected with the control adenovirus. The small tumors recovered from these three mice showed extensive regions of cell necrosis, which did not occur in the larger tumors from the control mice (Fig. 9). All of the mice appeared healthy at the end of this experiment, but a morphological examination of the dissected mice revealed damage to the liver and spleen in the five mice injected with the adenovirus encoding the *mfVIIasm* immunoconjugate. Histological examination of the liver and spleen showed that many of the liver cells were enlarged, and the spleen was extensively infiltrated with erythrocytes. Enlarged liver cells also occurred in a previous experiment after three injections of the adenovirus encoding the *mfVIIasm* immunoconjugate, but the spleen was normal, indicating that the defects in the spleen developed in the course of the last two injections. One of the mice also had a subdural brain hemorrhage, which did not occur in other mice from this experiment or any of the previous experiments. It is uncertain whether this defect was induced by the binding of the *mfVIIasm* immunoconjugate to

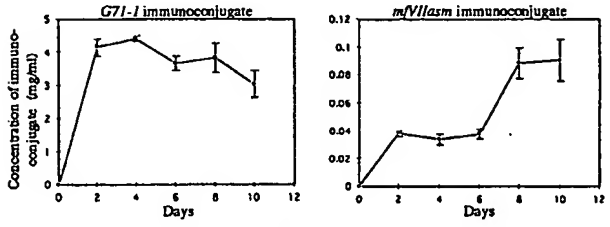

FIG. 4. Concentrations of the *G71–1* and *mfVIIasm* immunoconjugates in the blood of SCID mice after i.v. injections of the adenovirus encoding each immunoconjugate. The mice were injected on days 0 and 7 with 2×10^{11} adenovirus encoding the *G71–1* immunoconjugate or with 4×10^{11} adenovirus encoding the *mfVIIasm* immunoconjugate. The concentration of the encoded immunoconjugate in the blood was determined by ELISA. Each point is the average of the concentration for the five mice in each group.

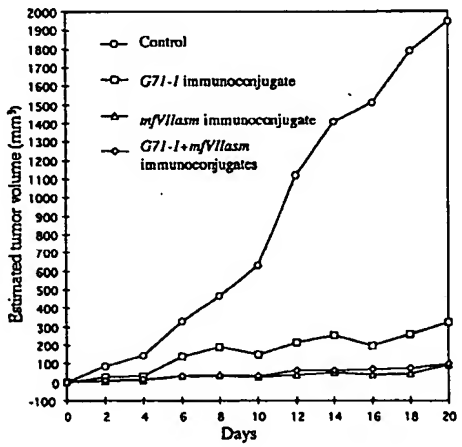

FIG. 5. Inhibitory effect of the *G71–1* and *mfVIIasm* immunoconjugates on the growth of a TF2 xenograft. For each curve five SCID mice were injected s.c. with 5×10^5 TF2 cells. When the xenografts had grown to a palpable size, the mice received tail vein injections on days 0, 7, and 14 of the adenoviruses indicated. The amount of adenovirus injected was 4×10^{11} for the control, 2×10^{11} for the adenovirus encoding the *G71–1* immunoconjugate, and 4×10^{11} for the adenovirus encoding the *mfVIIasm* immunoconjugate. The estimated tumor volumes are the averages for the five mice in each group.

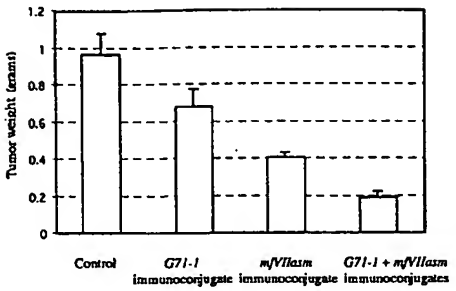

FIG. 6. Tumor weights of the xenografts from the experiment reported in Fig. 5. The xenografts were dissected from the mice on day 20, which was 6 days after the last injection of adenovirus. The bar heights are the average weights for the five mice in each group.

TF expressed in the brain vasculature or occurred spontaneously.

DISCUSSION

A SCID mouse xenograft model of human melanoma was used to test the therapeutic potential of an immunotherapy procedure designed to target both the tumor vasculature endothelial cells and tumor cells for cytolysis by the host immune system. The procedure involved systemic delivery to SCID mice of two immunoconjugates, each composed of a tumor-targeting domain conjugated to the Fc region of a human IgG1 heavy chain, forming a homodimeric molecule similar to a Camelid heavy-chain antibody (19). For one type of immunoconjugate, the tumor-targeting domain was the human scFv molecule *G71–1* that binds to the melanoma antigen MCSP (1, 4) expressed by the melanoma cells in the xenografts. For the other type of immunoconjugate, the tumor-targeting domain was a mfVII molecule that binds specifically and tightly to TF, both to mouse TF expressed by the tumor vasculature endothelial cells and to human TF expressed by the melanoma cells in the xenografts. To decrease the risk of disseminated intravascular

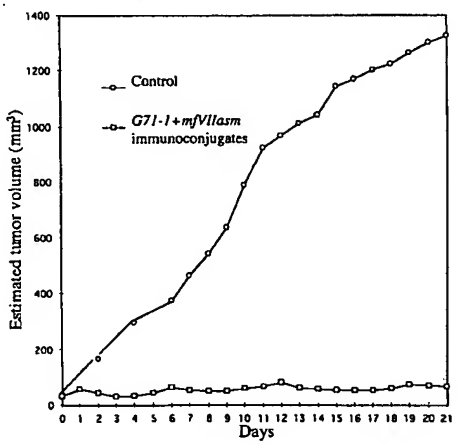

FIG. 7. Inhibitory effect of the *G71–1* and *mfVIIasm* immunoconjugates on the growth of a larger TF2 xenograft. Each mouse was injected s.c. with 5×10^5 TF2 cells, and the xenografts were allowed to grow to an estimated tumor volume of 50 mm^3 on the skin surface (day 1). A mixture of 2×10^{11} adenoviruses encoding the *G71–1* immunoconjugate and 7×10^{11} adenoviruses encoding the *mfVIIasm* immunoconjugate was injected into the tail vein of five mice on days 1, 6, 12, and 19. As a control five mice were injected with 4×10^{11} adenoviruses that did not encode an immunoconjugate. The estimated tumor volumes are the averages for the five mice in each group. One of the mice injected with the adenoviruses encoding the immunoconjugates was found dead on day 17; the estimated tumor volumes on subsequent days are the averages for the remaining four mice.

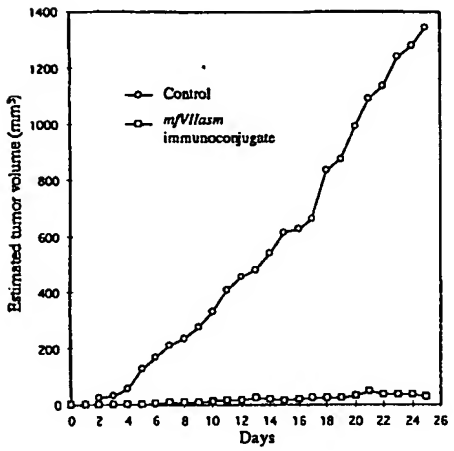

FIG. 8. Inhibitory effect of the *mfVIIasm* immunoconjugate on the growth of an LXSN xenograft. The mice were injected s.c. with 5×10^5 LXSN cells, and when the xenograft had grown to a palpable size (day 0) five mice were injected with 9×10^{11} adenoviruses encoding the *mfVIIasm* immunoconjugate, and five mice were injected with 4×10^{11} control adenoviruses. Additional injections were done on days 7, 13, 21, and 24, and on day 25 the mice were dissected for morphological and histochemical examination. The estimated tumor volumes are the averages for the five mice in each group.

coagulation that might result from the binding of a fVII immunoconjugate to TF, an active site mutation was introduced into the mfVII targeting domain (*mfVIIasm*), inhibiting the proteolytic activity required to initiate the blood coagulation pathway.

An earlier *in vitro* study showed that the *G71–1* immunoconjugate mediates cytolysis of cultured human melanoma cells by natural killer (NK) cells and complement (1). Because SCID mice retain the capacity to produce functional NK cells and complement, the immunoconjugates also could mediate cytolysis of the targeted tumor cells and vascular endothelial cells of a human melanoma xenograft growing in SCID mice. Systemic delivery of the immunoconjugates to SCID mice was achieved by tail vein injections of a replication-defective adenoviral vector encoding the immunoconjugates, which were secreted into the blood for at least 1 week after each injection. The mice first were injected s.c. with a human melanoma cell line that expresses either a low or high level of TF, and the resulting xenograft was allowed to grow into a small (≈5 mm^3) or larger (≈50 mm^3) tumor before starting injections of the adenoviral vectors. Further growth of all the xenografts was prevented for the 3- to 4-week period of the experiments by multiple injections of the adenovirus encoding the *mfVIIasm* immunoconjugate, administered separately or together with the adenovirus encoding the *G71–1* immunoconjugate; in some of the mice the xenograft completely regressed. In the control mice, which were injected with an adenovirus that did not encode an immunoconjugate, the average volume of the xenografts increased by a factor of about 25 during the same period. In the mice receiving five injections of the adenoviral vectors encoding the immunoconjugates, many of the liver cells were enlarged and the spleen was infiltrated with erythrocytes. The defects were not caused by the secreted immunoconjugates, which do not bind to the liver or spleen cells. The primary cause probably is the continuous high-level synthesis of the encoded immunoconjugates by the liver cells, which are the mouse cells predominately infected by i.v. injected adenoviral vectors (18). The enlarged liver cells could have produced an increased blood pressure in the spleen, causing blood vessels to rupture. If these defects also can occur in a clinical setting, the problem might be corrected by changing the dose, schedule, or route of injection for the

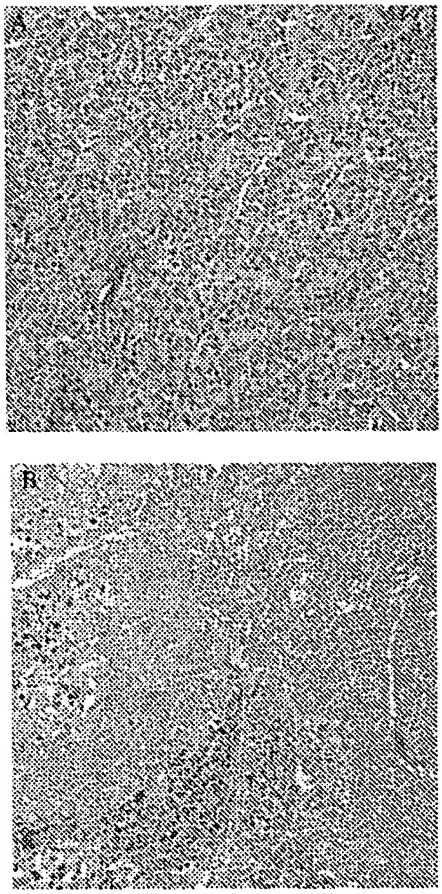

FIG. 9. Histochemistry of the LXSN xenografts from the experiment reported in Fig. 8. The xenografts were dissected on day 25 and embedded in paraffin, and sections were stained with hemotoxylin + eosin. (*A*) Xenograft from a control mouse injected with the adenovirus that does not encode an immunoconjugate. (*B*) Xenograft from a mouse injected with the adenovirus encoding the *mfVIIasm* immunoconjugate. Magnification: ×245.

adenoviral vectors, or by using a different type of vector. Another possible option is to administer the immunoconjugates directly as proteins.

Although the immunoconjugate concentration in the blood of SCID mice injected with an adenoviral vector was about 100-fold higher for the *G71–1* immunoconjugate than for the *mfVIIasm* immunoconjugate, the inhibitory effect on a human melanoma xenograft nevertheless was stronger with the *mfVIIasm* immunoconjugate. A key advantage of the *mfVIIasm* immunoconjugate is the binding that occurs to tumor vascular endothelial cells as well as to tumor cells, in contrast to the *G71–1* immunoconjugate that binds only to melanoma cells. The binding to the tumor vasculature should be tumor specific, because TF is not expressed by the normal vasculature. Although TF is expressed by several other normal tissues, such as brain, lung, and kidney glomeruli, these TF molecules are not accessible to endogenous fVII or a fVII immunoconjugate because the blood vessel walls form a barrier separating larger blood components from adjacent cells. However, tumor blood vessels are leaky (14), allowing access to TF expressed by tumor cells. Thus, a human *fVIIasm* immunoconjugate could be an effective therapeutic agent for a broad spectrum of human tumors expressing TF on the vascular endothelial cells and tumor cells. The therapeutic efficacy of a human *fVIIasm* immunoconjugate could be enhanced by also administering a human scFv immunoconjugate that binds to a tumor target other than TF.

In considering a clinical test of the immunoconjugates, which might require maintaining an adequate titer in the patient's blood for a prolonged period, an immune rejection response to the immunoconjugates and/or the adenoviral vector could be a potential obstacle. Because the tumor-targeting and Fc effector domains of the immunoconjugates are derived from human sources for clinical protocols, the immunoconjugates should be tolerated by the human immune system. Although it was possible to use the adenoviral delivery system for repeated injections in SCID mice, an adenovirus might be too immunogenic in patients for this purpose. To avoid immune rejection of the vector, a nonimmunogenic vector could be substituted for the adenovirus, or the immunoconjugates could be administered directly as proteins.

We are grateful for the contributions of our colleagues, Dr. Michael Bromberg, who generously provided the human melanoma cell lines LXSN, TF2, and LXSN/VEGF, and Dr. Baiyang Wang, who provided advice and assistance for the experiments. The mouse autopsies and examinations were done by Gordon Terwilliger. Dr. Albert Deisseroth, Section Chief of Medical Oncology, Yale University School of Medicine, provided essential resources and facilities for producing adenoviral vectors. This project was supported by U.S. Public Health Service Grant PO1 HL29010 and a generous gift from private donors (S. L. Misrock and A. M. Fox).

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